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STUDY OF CONGENITALLY MISSING SECOND PREMOLARS AND SPACE FACTORS IN THE ARCHES[1]

CLARENCE P. OLIVER, PETER J. BREKHUS, AND GEORGE MONTELIUS

The Dight Institute of Human Heredity and the School of Dentistry, University of Minnesota, Minneapolis, Minn.

The theory that human dentition is passing through a transitional stage leading to a decrease in the number of teeth is supported by most odontologists. Recently, Dahlberg (2) discussed this tendency and referred to the general trend toward a reduction in size, form, and number of teeth in human beings. The causal factors associated with this deficiency in the dental arches is difficult to identify.

Several theories which have been presented, such as those by Montagu (3), Schultz (5), and others, seem to fit special cases but do not account for the congenital absence of teeth located in the premolar region of the arches. Mere chance alone does not explain the failure of some types of teeth to develop. In an earlier publication (1), the authors called attention to a definite pattern or regularity with which certain teeth failed to develop in 184 persons. They reported that the teeth most frequently congenitally missing were the upper second incisors, second premolars, and third molars. Any other tooth may on occasion be congenitally absent, but except for the lower first incisors the frequency is very low. Since that report, 232 new cases of congenitally missing teeth have been collected and studied. In tabulating the new data we find that the pattern of the congenital absence of teeth remains the same. The upper second incisors, second premolars, and third molars which are most often absent have also been found to be the most variable in pattern and size (6). It seems justifiable, therefore, to assume that some factor or factors control the variation in the development of these tooth buds.

The authors of this report have suggested in a previous publication (1) that arch size and congenital absence of teeth seem to be separate entities. This contention is also borne out by our study of missing second premolars which is reported in the present paper. All interpretations are based upon examinations of the patients with a study of dental models made from impressions of the arches and complete oral radiographs of those persons.

DATA

In order to determine the exact proportion of patients of the clinic who have congenital dental defects, one operator recorded the anomalies observed in 3699 persons whom he examined in a 21-month period. Among these patients, 176, or 4.8%, lacked 1 or more teeth or had peg-shaped or supernumerary teeth.

[1] This study was made possible by aid from the Committee of Human Heredity of the National Research Council and by supplemental aid from the Graduate School, the School of Dentistry, and the Dight Institute of the University of Minnesota. Received for publication August 17, 1945.

Seventy-seven, or 2.1%, of the patients had anomalies of the upper second incisors. Eighty-seven, or 2.4%, were found to have anomalies of the second premolars in 1 or more of the quadrants. These incidences are higher than those reported by us earlier (1), and a probable explanation of the low frequencies was given at that time. The general pattern agrees with the one discussed in that report.

Among 427 persons now in our records, who have congenital anomalies of the teeth, 162 show anomalies of the second premolars, of which 153 are reported in this study. These 153 persons failed to develop a total of 330 second premolars. The number lacking in each quadrant is shown in Table I. In the upper arch 62 were absent in the right portion and 77 in the left. In the lower arch the respective numbers were 87 and 104.

TABLE I

Number of missing permanent second premolars and retained deciduous second molars in 153 persons

	MISSING PERMANENT IN QUADRANTS		RETAINED DECIDUOUS IN QUADRANTS	
	Right	Left	Right	Left
Upper	62	77	23	17
Lower	87	104	35	40
Totals	330		115	

In this same group of persons, 64 had one or more deciduous second premolar at the time they were examined, the number of retained deciduous teeth being 115. The number retained in each of the 4 quadrants is shown in Table I. In the upper arch, 23 were present in the right and 17 in the left part of the jaw. In the lower arch, 35 remained in the right and 40 in the left.

MISSING PREMOLARS AND SPACES

One of the important functions of the deciduous second premolar is to retain space for its successor, the permanent second premolar. With the early loss of the deciduous tooth, very often the permanent first molar will erupt mesially. When the permanent second premolar is ready to erupt, the space has been filled by the first molar. A rearrangement of the entire dental arch often becomes necessary in order to recreate space for the second premolar. Very often the second premolar is extracted as the lesser of two evils. In persons who never develop the second premolar, the closing of the space may lead to a perfect alignment of the teeth similar to the condition shown in *fig. 3*.

The general contention has been that lack of space in or decrease in size of the arches is responsible for the congenital absence of teeth. With this in mind we found it of special interest to observe if loss of space may be a contributing factor in the disappearance of the second premolars in man's dental formula. The data are shown in Table II.

Among the 153 persons with congenitally absent second premolars, 64 had 1 or more of their deciduous molars present with ample space for a permanent tooth

TABLE II

Proportion of persons retaining deciduous teeth or spaces in second premolar area

	DECIDUOUS TEETH			
	Present	Absent, but space ample	Absent, with slight space	Absent, with no space
Number of persons.....	64	32	21	36
Per cent.....	41.8	20.9	13.7	23.5

Fig. 1. Absent 5 with retained V in upper left arch of male, 20 years old.

Fig. 2. Absent 5 with retained V in lower left arch of same patient as *fig. 1.* The patient retained the V's in all 4 quadrants.

Fig. 3. Absent 5 with space occupied by 6 in lower right arch of a female, age 20 years. The 5's are absent in all 4 quadrants and, spaces were closed except for slight space in lower left.

Fig. 4. Absent 5 with ample space in lower left arch of a female, 19 years old. Lower right arch had a retained V. Upper arches had the 5's.

to erupt (figures 1 and 2). Although the other 89 persons had lost all their deciduous molars, 32 still had ample space for a permanent premolar in 1 or more of the quadrants (*fig. 4*). In 21 cases, the retained space was less than enough

to accommodate the permanent tooth and in 36, the space between the first premolar and the first molar was closed. In these persons, therefore, 96 out of 153, or approximately 63%, had ample space for 1 or more premolars which did not develop. Only 24% of the persons had no space between the first premolar and the first molar. In some instances one person had a retained deciduous tooth or full space in 1 quadrant and only a partial space or no space at all in another quadrant.

DISCUSSION

The evidence reported in this study substantiates the belief that lack of space in the arches is not a factor in the congenital absence of the second premolars. The greater proportion of persons who lacked those teeth retained the larger deciduous teeth or had ample space for the permanent teeth in 1 or more of the quadrants. A single individual who congenitally lacks all 4 permanent second premolars sometimes will retain a deciduous tooth in one quadrant, have ample space in another, and lack space in still another. Where the deciduous tooth is not retained, the amount of space between the first premolar and the first molar probably is determined by the age at which the deciduous second molar was lost. An effect of age at extraction of first molars on the amount of space between the premolars has been reported by Salzmann (4).

Accident alone cannot account for the congenital absence of teeth. If that were true, all tooth germs should be affected with equal frequency. We have ample evidence that this does not occur. Certain types of teeth are seldom congenitally absent. Others, namely the third molars, upper second incisor, second premolars, and to a lesser extent lower first incisors, are frequently missing. This pattern occurs with a regularity which suggests an orderly process associated with the congenital loss of the teeth.

The factor responsible for the congenital absence of one type of tooth will probably explain most of the absences of the other types as well. Second premolars, which are in the midportion of the arches quite removed from anatomical structures associated with the incisors or third molars, are missing almost as frequently, if not as frequently, as the upper second incisors. It seems probable, therefore, that the factor responsible for the congenital loss of teeth controls the development of the dental buds, possibly the rate of development, regardless of the position in the arch. Certain buds are more likely to be affected by this modified development than are other buds.

SUMMARY

A brief report is made on the incidence of congenitally missing teeth in 3699 persons. The conditions observed in persons lacking second premolars are discussed.

Among 153 persons with congenitally missing second premolars, 330 of the premolars failed to develop. In these persons, 115 deciduous second molars were present at the time of examination.

Deciduous teeth were present or ample space still remained in 1 or more quadrants of 96 of the 153 persons who lacked second premolars. Of the others, 36 had no space and 21 had only slight space between the first premolar and first molar.

It is concluded that lack of space in the arches cannot account for the congenital loss of second premolars. It is suggested that the factor causing the loss of these teeth probably controls the development of the dental buds, and that the same factor effects the loss of the other kinds of teeth which are frequently missing.

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ELECTRON MICROSCOPE STUDY OF THE MECHANISM OF FLUORINE DEPOSITION IN TEETH[1]

CHARLES H. GEROULD

The Dow Chemical Company, Midland, Mich.

INTRODUCTION

Recent work on tooth structures with the electron microscope, using surface replica techniques (1, 2), has indicated that this new research tool may be used profitably in dental research. The indications are that this instrument may be used not only in studying the fundamental histology of teeth, for which it seems well suited, but for pathological studies as well.

The widespread and rapidly increasing interest in the effect of fluorine upon dental caries resistance has led us to speculate as to whether the electron microscope might conceivably be used in gaining a better understanding of the action of fluorine upon dental tissues. It was thought that possibly the electron microscope, with its tremendous resolving power, might show microstructural differences between normal and fluorosed teeth which would throw more light on the fluorine-dental caries relationship.

The abundant fluorine literature of which there have been excellent reviews (3, 4, 5) has revealed that population groups using optimum-fluoride-containing domestic waters during the period of tooth calcification show a lowered caries incidence. This inverse relationship between fluoride concentration and dental caries holds true whether the fluoride content of water supplies is such that considerable severe mottling occurs or whether it is such that the mottling ceases to exist except sporadically and in the very mildest forms.

Individuals who were exposed to fluorides in domestic waters during the critical period of tooth calcification, and afterward changed to a fluoride-free water supply, continued to have an increased caries resistance (6). In the opposite case where individuals who were exposed to fluoride-free waters during tooth developmental periods and later left such waters for those containing fluorides, no increase in dental caries resistance or macroscopic mottling occurred, although the fluoride (F) concentration may have been as high as 14 ppm. and the exposure for many years (7).

Analytical data on enamel and dentin shows the fluorosed or macroscopically mottled teeth to contain 3 to 5 times as much fluorine as normal teeth (8), and this fluorine content is directly proportional to caries resistance (9). Our chemical analytical data has confirmed these figures. Since completely unerupted teeth from endemic areas often show macroscopic mottling, it appears probable that this fluorine arrives in the teeth via systemic ingestion and not surface absorption.

A different angle of the fluorine-dental caries relationship is the topical application of soluble fluorides to the tooth surface *in situ*. Knutson and Armstrong

[1] Received for publication August 20, 1945.

(10) have reported that 7 to 15 applications of an aqueous 2% solution of sodium fluoride to children's teeth caused a 40% decrease in caries over a period of 1 year. During such topical applications no visible indications of dental fluorosis have been reported though fluorine is incorporated into the enamel, at least on the surface (11, 12), and this takes place without appreciable systemic ingestion (13).

The facts presented in the preceding paragraphs indicate that there is a difference in the mechanism of fluorine deposition in the case of the fluorosed teeth resulting from systemic ingestion, as compared to the teeth treated by topical application. In this work, combined use was made of X-ray diffraction, electron diffraction, chemical analysis and electron microscopy in an attempt to prove that different mechanisms of fluorine deposition really exist and to learn as much as possible concerning these mechanisms. The literature on this subject has been rather meager and inconclusive, indicating the need for research in this field.

The electron microscope techniques used in this work have been described in detail in the literature (2). Briefly, the techniques consist of sectioning the extracted tooth, polishing, lightly etching with hydrochloric acid to bring out the structural details in relief and then preparing the replica. The replica technique, known as the polystyrene-silica technique (14), consists of a procedure whereby a polystyrene impression is first made of the prepared tooth surface. This plastic is then separated from the tooth, and silica evaporated in a very thin film upon the plastic moulding. This silica film is scribed into squares, removed from the plastic with a suitable solvent and used directly in the transmission electron microscope as the specimen.

This work was considerably limited by the difficulty in obtaining teeth showing indications of dental fluorosis. The teeth obtained all showed macroscopic mottling. Our efforts to obtain fluorosed teeth, which showed no mottling, were to no avail. The history of the fluorosed teeth used was either not available or largely incomplete.

This paper is based upon very limited data. The work is quite preliminary in nature. The author is unable at present to continue this work to its logical conclusion, but feels that the available data should be published at this time. It is hoped that others may find these research tools and techniques useful in continuing this work for there appears to be need for an exhaustive study on this subject.

MICROSTRUCTURAL COMPARISONS

Comparisons between the polished and etched dentin of normal teeth and that of fluorosed teeth show a difference which seems to be of significance in this study. This difference is entirely one of the fineness of detail to be found in the etched matrix between the dentinal tubules, the fluorosed dentin showing the much finer detail. The appearance of this background structure varies somewhat from tooth to tooth and from region to region within a tooth; but, regardless of these variations, the particle dimensions remain in the same order of magnitude, roughly 0.01 microns for the fluorosed dentin to 0.1 microns for the normal dentin. Structural details such as these are far beyond the resolution

of the best light microscopes. These differences can be observed in the electron micrographs of *figs. 1a* and *1b*, which show normal and fluorosed dentin with the dentinal tubules sectioned nearly transversely in both cases. The black area partially circling each individual tubule is due to a sharp elevation change in the silica film.

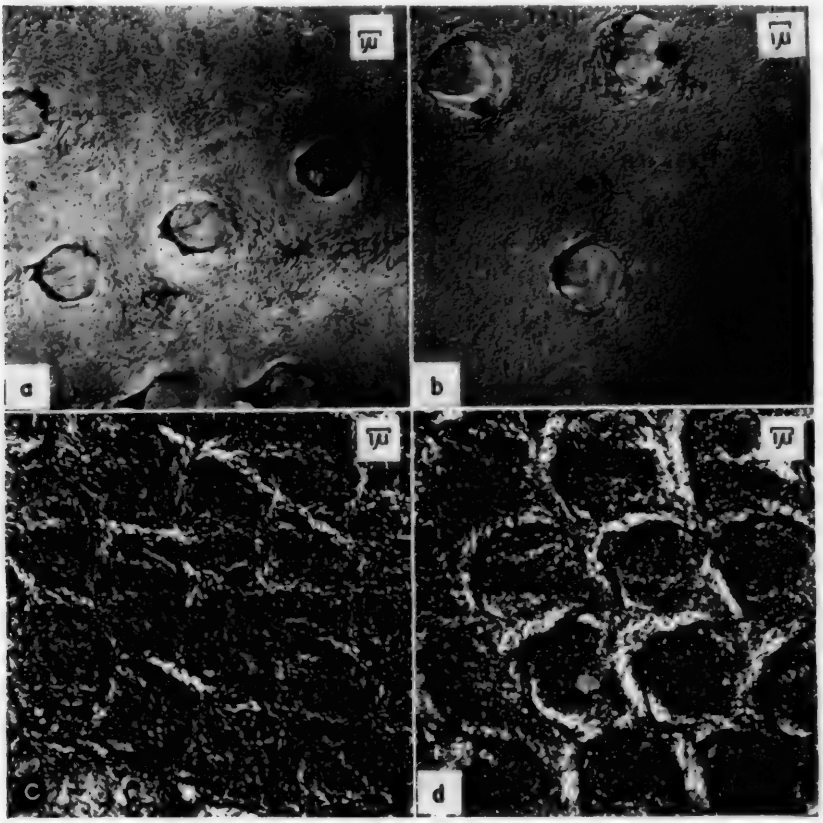

Fig. 1. Comparison between (a) normal and (b) fluorosed dentin etched with HCl and (c) normal and (d) fluorosed enamel etched with HCl. (Orig. mag. ×6500.)

The fluorosed tooth from which micrograph 1b was obtained came from Lamesa, Texas where domestic waters contain about 5 ppm. fluoride (F). The dentin differences illustrated here have been observed in 12 normal teeth compared to 5 fluorosed teeth. Among the latter was a completely unerupted molar from Colorado Springs, Colorado (2.6 ppm. F in municipal waters) which showed severe mottling. The electron micrographs from the dentin of this tooth showed the fine background structure typical of fluorosed tooth structures although the tooth had never been in direct contact with the fluoride-containing waters.

Microstructural comparisons between the polished and etched enamel of various teeth are a much more difficult task. Normal enamel when polished and etched lightly with hydrochloric acid gives a surface which is exceedingly rough on a very fine scale. This apparently is due to considerable variations in acid solubility of the different enamel constituents. The roughness of this etched surface makes it necessary to dissolve the enamel section away from the polystyrene moulding during the replica process. This generally results in leaving residues on the polystyrene surface which makes it very difficult to obtain clean silica replicas. However, in the few cases in which fairly good results were obtained it has appeared possible that the etched fluorosed enamel also showed a finer etch-structure than did the normal enamel. This difference is very difficult to evaluate since the normal enamel structure is so rough, and consideration must always be given to the possibility of artifacts due to residues on the silica film.

An enamel comparison is illustrated in *figs. 1c* and *1d* in which the enamel rods have been sectioned transversely. No obvious differences have been observed concerning the interprismatic material between the enamel rods. Here again as with the dentinal tubules, the sharp elevation change between the enamel rod and the surrounding interprismatic material (due to differences in solubility in the hydrochloric acid etchant) show as dark regions. Organic residues from the interprismatic material may add to this effect.

Electron micrographs of the external surface of enamel, without etching, are quite varied in appearance, some showing a much rougher surface than others. These variations also appear from surface to surface in an individual tooth. The external enamel of several teeth of both the normal and fluorosed class were examined and the general observation is that the enamel surface of the fluorosed tooth tends toward a greater roughness on a microscopic scale though this is not always the case. In some cases, continuous long parallel lines or bands of rough structure have been observed on the surface of fluorosed teeth. These bands are roughly 10 microns wide and are spaced 50 to 100 microns apart which appear to coincide with the intersection of the bands of Retzius with the surface.

Fig. 2 shows a comparison between the (a) normal and (b) fluorosed external enamel. Micrograph (b) is from one of the bands of rough structure apparently corresponding to a stria of Retzius. The surface between these bands is much smoother as can be seen at the left edge of micrograph (b).

Since it was so difficult to obtain good replicas from etched enamel, an attempt was made to circumvent these difficulties by examining cleaved enamel structures without etching. This gave very good results as far as the quality of the replicas obtained was concerned, but gave no new light on fluorine deposition since normal and fluorosed teeth showed similar cleavage patterns. *Fig. 3* shows a comparison between the longitudinally cleaved and unetched (a) normal enamel and (b) fluorosed enamel. Notice that in both cases the fracture pattern shows a tendency for a rectangular shape. The darker horizontal bands observed especially in (a) show cleavages through the interprismatic material.

Cleavage sections were also obtained of dentin and though no significant differences could be observed between the unetched normal and fluorosed dentin, the

Fig. 2. Comparison between the unetched external enamel surface of (a) normal and (b) fluorosed teeth. (Orig. mag. ×6500.)

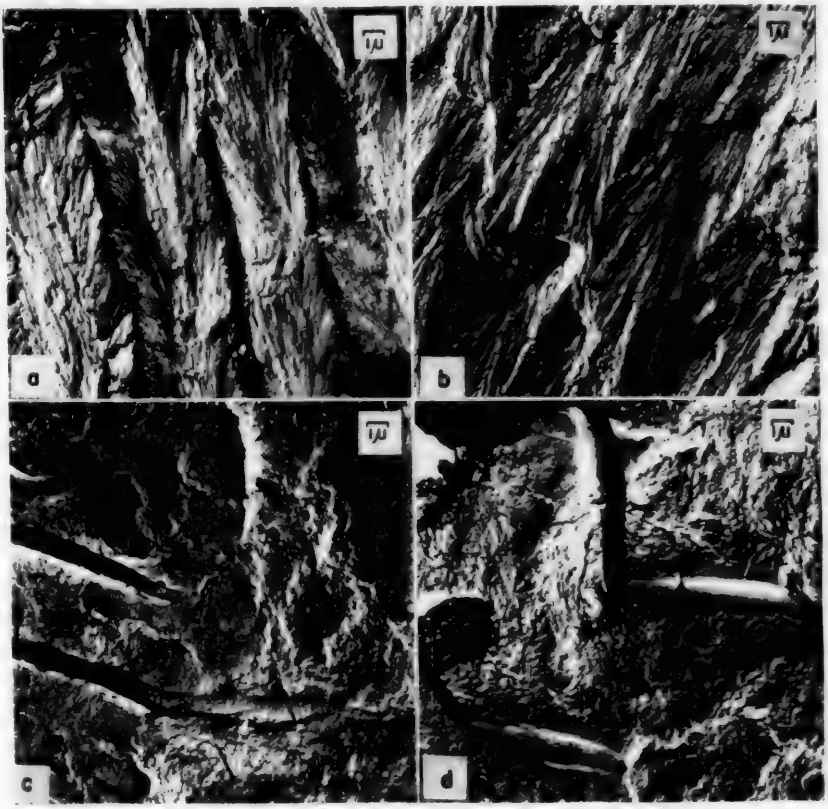

Fig. 3. Comparison between unetched cleaved enamel of (a) normal and (b) fluorosed teeth and unetched cleaved dentin of (c) normal and (d) fluorosed teeth. The latter micrographs show oblique section of dentinal tubules with Tomes fibers extending from tubule openings. (Orig. mag. ×6500.)

results were very interesting from a fundamental histologic viewpoint. In preparing replicas of the cleaved dentin it was found necessary to dissolve the tooth section away from the polystyrene moulding using 15 to 20% hydrochloric acid. The acid readily decalcified the tissue leaving the ends of isolated broken Tomes fibers clinging to the polystyrene replica. After silica had been evaporated upon the polystyrene replica and the polystyrene then dissolved from the silica replica film, the broken ends of the isolated Tomes fibers were left attached to the silica film. The electron micrographs shown in *Figs. 3c* and *3d* show the cleaved surface of the matrix dentin with oblique cleavage through the dentinal tubules. The actual isolated Tomes fibers can be seen extending from these dentinal tubules and appearing as dark strips.

It is seen that the electron density of these fibers (1 to 1½ microns in diameter) is sufficiently low to allow one to see through them, i.e., they are not opaque to the electron beam. This indicates that the isolated Tomes fibers as observed are not a solid fiber but rather a thin walled tube, apparently tightly lining the tubule wall and having a wall thickness of 0.1 microns or less, judging from electron transmission. A solid fiber of 1 micron diameter would be completely opaque to the electron beam. It has been suggested that this effect is due to artifact resulting from dehydration of a solid protoplasmic Tomes fiber during the replica procedure at which time the residue was deposited on the tubule wall as a thin film and held there by fibril branches. It is interesting to note that when dentin structure from an old elephant tusk was cleaved and a replica prepared in the same manner as was the replica of cleaved human dentin, the results gave isolated thin walled Tomes fibers which were so similar to those isolated from human dentin as to appear practically the same. The author presents this information without further comment since he feels unqualified to enter into the age-old controversy on the dentinal tubule.

TOPICAL APPLICATION OF FLUORIDES

A number of experiments have been performed in which aqueous fluoride solutions have been topically applied to normal extracted teeth. Generally the fluoride concentration and duration of application were much greater than those actually used in the topical applications reported in the literature. This was felt advisable in order to make the analytical data as definite as possible. The problem was made increasingly difficult since the electron and X-ray diffraction patterns for the various apatites (hydroxy, carbonate, fluor) believed to exist in teeth are so similar as to be practically inseparable.

Normal enamel was polished and etched lightly to give a surface such as shown in *fig. 1c*. The enamel was then immersed for about 1 month in a 4% aqueous solution of sodium fluoride and then washed and dried. A replica was prepared of the surface, which was generally very dirty, inasmuch as reaction products which had formed on the surface of the enamel adhered to the replica. However, some regions were clean, exhibiting a type of structure shown in *fig. 4a*. The outlines of the enamel rods can still be seen though an entirely different general structure exists. Both electron diffraction and X-ray diffraction show this surface to consist of calcium fluoride.

Normal enamel was polished and immersed for about 1 month in a 4% sodium fluoride solution. At the end of that time the enamel was washed, dried and etched for the usual etching time of 10 seconds in N/10 hydrochloric acid. Since macroscopically the surface appeared to be unchanged by the etching, a replica was made to check this. *Fig. 4b* shows this surface as having been unattacked by the etchant except in very localized regions. One of these pitted regions is shown in this micrograph with the unattacked surface surrounding the pit still showing the polishing scratches. This indicates that the deposition of calcium fluoride increases the acid resistance of the enamel.

In a case similar to that described in the preceding paragraph, the enamel of a tooth was polished and immersed for about one month in a 4% sodium fluoride solution. The enamel was washed and dried and then etched in an attempt to

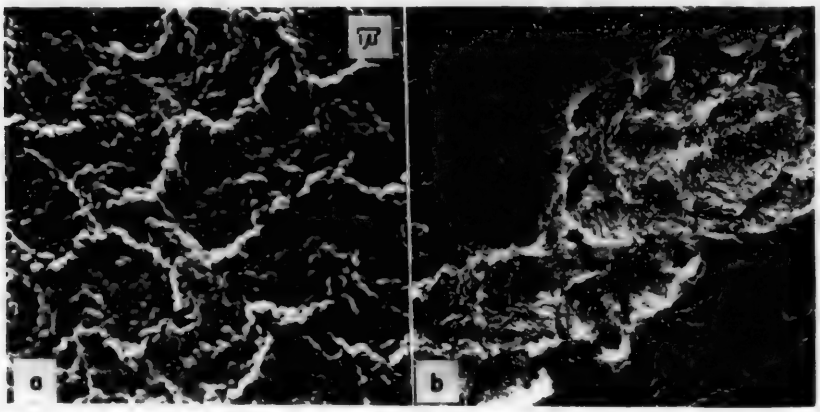

Fig. 4. (a) Normal enamel, polished and etched + immersion in 4% sodium fluoride solution. (b) Normal enamel, polished + immersion in 4% sodium fluoride solution + ordinary enamel etch. Localized pit attack. (Orig. mag. ×6500.)

duplicate the usual depth of etch. It was found necessary to etch the treated enamel for 5 to 10 seconds with N/1 hydrochloric acid to give an etch which appeared to be of the same degree as the usual 10 second etch in N/10 hydrochloric acid. A replica of this freshly etched surface showed typical enamel with nothing resembling the type of fine structure occurring in fluorosed teeth in which the fluorine was incorporated by systemic ingestion.

The literature mentions the ease with which pulverized enamel picks up fluorine from aqueous fluoride solutions (12). During an investigation of this type of action, normal enamel with some dentin was pulverized with mortar and pestle to a fine flour. A sample of this enamel was dispersed in water, allowed to settle and the finer suspended material examined directly in the electron microscope. *Fig. 5a* shows the appearance of representative particles and aggregates of this untreated enamel. A further sample of this same pulverized enamel was added to a 4% sodium fluoride solution and allowed to stand overnight. The mixture was then agitated and after 2 hours of settling a sample of

the still suspended solids was removed, washed and examined directly in the electron microscope. *Fig. 5b* shows the appearance of this fluoride-treated enamel.

X-ray diffraction analysis shows the untreated pulverized enamel to consist of 100% apatite and the fluoride-treated enamel to consist of 80 to 90% calcium fluoride with only 10 to 20% of apatite remaining. *Micrograph 5b* shows this calcium fluoride as having a granular structure with a considerably reduced electron density from that of the original apatite, as indicated by the transmission of electrons through the outer parts of the particles. Since electron diffraction results on the treated enamel shown in *5b* indicated 100% calcium fluoride, it is concluded that this electron transparent coating on the particles is also calcium fluoride.

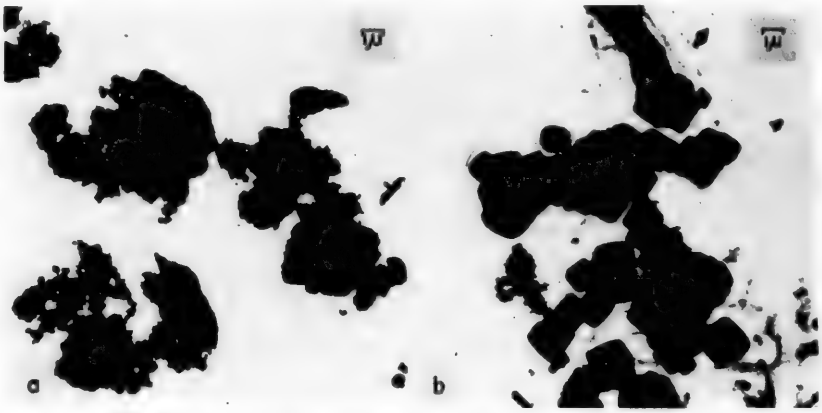

Fig. 5. Comparison between (a) normal pulverized enamel and (b) normal pulverized enamel after NaF treatment. (a) Apatite, (b) Largely CaF_2. (Orig. mag. ×6500.)

Other tests on pulverized enamel have shown that a 2% aqueous solution of sodium fluoride will deposit sufficient calcium fluoride in 30 minutes at 35 to 40°C. to be recognizable as such by means of both X-ray and electron diffraction. The depth of penetration of the fluoride during topical applications was not determined though it appeared that particles as much as 5 to 10 microns in diameter were largely converted to calcium fluoride.

MECHANISM OF FLUORIDE DEPOSITION

The literature data previously mentioned has shown that fluorine systemically ingested during tooth calcification enters into the tooth structure increasing by several times the fluorine content of both enamel and dentin. Electron micrograph comparisons of cleaved but unetched normal and fluorosed enamel and dentin show no significant differences, indicating that the dispersion of the fluorine is such that it does not greatly influence the fracture pattern of the normal apatite structures. However, when the fluorosed enamel or dentin is etched with hydrochloric acid a new fine type of background structure appears

indicating an exceedingly fine dispersion of a new mineral phase more resistant to the hydrochloric acid etchant than the hydroxy and carbonate apatites normally found in teeth.

Theoretically, it would seem probable that any new mineral phase appearing in a fluorosed tooth would consist of either calcium fluor-apatite or calcium fluoride. These two minerals with calcium hydroxy-apatite were roughly compared as to their relative solubilities in N/1 hydrochloric acid. The tests were made by adding 0.1 gram of the pulverized mineral to 50 ml. of acid for 2 hours at room temperature and determining the loss in weight. The data obtained was as follows:

Ca-hydroxy-apatite	80% loss in weight in N/1 HCl after 2 hours
Ca-fluor-apatite	56% loss in weight in N/1 HCl after 2 hours
Fluorite (calcium fluoride)	Negligible loss in weight in N/1 HCl after 2 hours

A severely fluorosed tooth was sectioned and the internal enamel polished and etched with hydrochloric acid as was usual in sample preparation. Judging from past experience the surface should have then been quite similar to *fig. 1d* with considerable very fine structure projecting in relief from the surface. This etched surface was then analyzed by electron diffraction using the reflection method whereby electron transmission was obtained through surface projections whose dimensions were in the order of 0.03 microns or less. The results showed a strong pattern of apatite with not the slightest trace of the calcium fluoride lines which certainly would have been very strong if any of this practically insoluble compound had been present. This method of analysis could be expected to be sensitive to even very minute quantities of the insoluble calcium fluoride since it would project outward from the etched surface where electrons would be diffracted by it.

It seems reasonable to believe that since calcium fluoride was not the projecting fine structure on the etched enamel, it might be the calcium fluor-apatite which is also less soluble than the ordinary apatite constituents of tooth enamel and would, therefore, be projecting in relief following etching. The electron diffraction analysis also showed some unidentified material projecting up from the etched surface. This could be either an actual mineral complex in the enamel or possibly a reaction product resulting from the action of the etchant.

The evidence for the formation of calcium fluoride during topical application of soluble fluorides to dental tissue has been covered quite thoroughly in the preceding section. The data indicates that the fluorine enters the dental tissue not by means of the physical process of adsorption as mentioned by Volker, Hodge, Wilson and Van Voorhis (12), but by the chemical process of double decomposition between the apatite and the soluble fluoride. In the case of sodium fluoride, the end products would probably be insoluble calcium fluoride, a soluble sodium phosphate and possibly some sodium hydroxide. The experiments with the pulverized enamel indicate that at least part of the soluble reaction products are leached out during the washing process. Electron diffraction of the pulverized enamel shows no constituents other than calcium fluoride near the surface of the particles. Undoubtedly soluble reaction products do still exist

inside of the particles of enamel but their concentration is not sufficiently high to enable identification by X-ray diffraction.

The increased acid resistance of enamel following topical application of a soluble fluoride and the probable resistance of all fluorosed teeth (15) are factors which certainly cannot be completely disregarded in any explanation of the decreased caries incidence which accompanies the entrance of fluorine into tooth structures. However, the author does not wish to give the impression that he feels that the acid solubility is necessarily the major factor. Rather, it seems that the reduced caries incidence is due to several factors, including both the acid solubility and the various effects of the fluorine upon the oral *Lactobacillus acidophilus* or other acidogens.

It has appeared difficult, in the past, to correlate the mechanism of caries reduction in the case of systemically ingested fluorides with that occurring with topical applications of fluorides. No single hypothesis has seemed to fit both cases. However, there are 2 significant things which are common to both. These are: (1) that fluorine is incorporated into the tooth structure in both cases; and (2) that the 2 fluoride compounds formed thereby are both more acid resistant than the normal apatite structures of teeth. In the light of this, a possible general mechanism explaining the fluorine-dental caries relationship might be as follows: (1) The oral *L. acidophilus* or other acidogens form acid which attacks the tooth surface preferentially, dissolving the more soluble apatites, and leaving the less soluble fluoride compounds concentrated on the surface. (2) This dissolution of the tooth surface by the acid-forming bacteria produces a solution containing progressively higher concentrations of fluoride ions *at the point of attack* and underneath any bacterial plaques present. The result is a high concentration of fluoride ions at the point of attack even though the fluoride concentration in the saliva may remain so low as to be considered negligible. (3) This progressive concentrating of fluoride ions will continue until the concentration is such that inhibition of acid production and bacterial growth results (16). Equilibrium conditions are thus established which are not conducive to active dental caries.

An analogy can be made to barnacle control on ships. The copper salts used in hull coatings are not readily soluble. Their activity, therefore, cannot be ascertained by analyzing the ocean near the hull, which is comparable to analyzing saliva for fluorine to determine if the fluorides in the teeth and oral cavity are capable of limiting the *L. acidophilus*. However, when a barnacle attaches itself to the treated hull and commences eating, excreting, etc., it finds locally a sufficient concentration of the ions of the copper salt to discourage its growth. It appears possible that something of a similar nature occurs to the *L. acidophilus*.

CONCLUSIONS

1. The hydrochloric acid etched dentin and enamel from fluorosed teeth exhibiting mottled enamel show a difference in microstructure from that of normal teeth. This difference is in the fineness of the background structure, the fluorosed dentin and enamel exhibiting the much finer detail.

2. Cleaved enamel and dentin structures of fluorosed and normal teeth (without etching) show no significant differences.

3. The external enamel surfaces of fluorosed teeth (without etching) show a tendency toward greater microscopic roughness than that of the normal teeth.

4. The experimental evidence indicates that fluorine entering into a tooth during the formative period or period of calcification is laid down, not in the form of calcium fluoride, but probably in the form of acid resistant calcium fluorapatite, as a fine dispersion throughout the hydroxy and carbonate apatites.

5. The experimental evidence indicates that fluorine entering into a tooth following the completion of calcification, is laid down in the form of acid resistant calcium fluoride.

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THE EFFECT OF SODIUM FLUORIDE ON THE SOLUBILITY OF CALCIUM PHOSPHATE, TOOTH ENAMEL AND WHOLE TEETH IN LACTIC ACID[1]

JAMES J. RAE AND CHARLES T. CLEGG

Chemistry Department, University of Toronto, Toronto, Canada

Earlier experiments of Box and Hodge (1) indicated that the addition of small amounts of sodium fluoride to lactic acid solutions in which whole teeth were suspended had the effect of inhibiting decalcification. In an attempt to gain additional insight into the chemical aspects of this mechanism under more carefully controlled conditions, the following experiments were performed.

EXPERIMENTAL

When whole teeth were suspended in 1% lactic acid solutions, buffered with potassium hydrogen phthalate and adjusted to a pH of about 3.6, marked decalcification occurred in 24 hours at 37°. No decalcification was apparent, however, when sodium fluoride was added under the same conditions in concentrations varying from 0.2 to 0.5%.

Tooth enamel was prepared according to the method of Manly and Hodge (2) and this material was used in an attempt to overcome individual variations in teeth. On shaking purified powdered tooth enamel with: (a) 1% buffered lactic acid and (b) 1% buffered lactic acid to which 0.5% sodium fluoride had been added, both at pH 3.5, a marked difference in the weights of residue left in the tubes after 24 hours was observed and these weights showed no appreciable difference after an additional 24 hours shaking. The addition of a similar amount of sodium chloride had only a very slight effect on the decrease in the weight of the residue. Determinations of inorganic phosphate on the supernatant liquids were made colorimetrically by means of King's method (3). The results of this experiment are shown in Table I.

These phosphate values would indicate that even though the sodium fluoride decreases the apparent solubility of tooth enamel (as shown by the differences in decrease in weight of residue) the enamel is being dissolved, as indicated by the phosphate values, to the same or to even a greater extent in the tubes containing fluoride.

This experiment was repeated, substituting pure tri-calcium phosphate in place of the tooth enamel, in view of the fact that powdered tooth enamel is a very complex substance. The results obtained (Table II) were entirely analogous to those described above, viz., the addition of sodium fluoride decreased the apparent solubility of calcium phosphate to about one-third of its solubility in 1% lactic acid; while at the same time the inorganic phosphate in the supernatants of all tubes rose to approximately the same values. The wide differences in calcium values would indicate that the decrease in the weight of the residue

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TABLE I

Effect of sodium fluoride on the solubility of tooth enamel

SOLUTIONS USED	DECREASE IN WEIGHT OF RESIDUE IN MG.	MG. P PER 10 ML. OF SUPERNATANT
1% Lactic Acid	31.6	4.5
1% Lactic Acid + 0.5% NaF	5.6	6.4

TABLE II

Effect of sodium fluoride on the solubility of calcium phosphate

SOLUTIONS USED	DECREASE IN WEIGHT OF RESIDUE IN MG.	MG. P PER 10 ML. OF SUPERNATANT	MG. Ca PER 10 ML. OF SUPERNATANT
1% Lactic Acid	52.2	12.65	23.0
1% Lactic Acid + 0.2% NaF	13.4	7.56	7.46

TABLE III

Effect of ammonium oxalate on the solubility of tooth enamel and calcium phosphate

SOLUTIONS USED	TOOTH ENAMEL		CALCIUM PHOSPHATE	
	Decrease in wt. of residue in mg.	mg. P per 10 ml. of supernatant	Decrease in wt. of residue in mg.	mg. P per 10 ml. of supernatant
1% Lactic Acid	65.0	5.2	65.8	11.6
1% Lactic Acid + 0.5% $(NH_4)_2C_2O_4$	38.8	7.0	21.8	9.8

TABLE IV

Effect of sodium fluoride and sodium oxalate on the decalcification of whole teeth

SOLUTIONS USED	APPARENT DECALCIFICATION	MG. P PER 10 ML. OF SUPERNATANT
1% Lactic Acid	Marked	7.31
1% Lactic Acid + 0.5% NaF	None	0.55
1% Lactic Acid + 0.5% $Na_2C_2O_4$	None	1.35

TABLE V

Effect of higher concentrations of lactic acid on decalcification of teeth

TUBE NO.	SODIUM FLUORIDE, %	pH	LACTIC ACID, %	DECALCIFICATION	MG. PER 10 ML.	
					P	Ca
1	0.2	3.75	0.5	none	2.78	none
2	0.2	3.56	1.0	none	2.35	none
3	0.2	3.30	2.0	none	1.98	none
4	0.2	3.20	3.0	none	2.57	trace
5	0.2	3.09	4.0	none	2.42	none
6	0.2	3.00	5.0	none	4.07	trace
7	0.2	2.80	9.0	none	4.74	none
8	—	2.35	9.0	marked	42.27	35.9

may be due to the dissolving of the calcium phosphate and redeposition of the calcium as calcium fluoride.

If such were the case the addition of any salt whose cations formed an insoluble calcium salt (e.g. ammonium oxalate) should have a similar effect. It was found that the addition of ammonium oxalate did decrease the apparent solubility of calcium phosphate and tooth enamel in 1% lactic acid. The results are shown in Table III.

Whole teeth were suspended in the following buffered solutions: 1% lactic acid; 1% lactic acid plus 0.5% NaF; 1% lactic acid plus 0.5% sodium oxalate (all adjusted to pH 3.7). The results obtained are shown in Table IV.

The fact that the phosphate values in the fluoride and oxalate tubes are much lower than the values in the tubes where the teeth are undergoing decalcification and the fact that all the phosphate values increase slowly with time indicates a fundamental difference between this experiment and the experiments with powdered tooth enamel and calcium phosphate. This difference may be explained by the fact that the enamel on the tooth is an organized material with much less surface exposed than powdered tooth enamel or calcium phosphate and so the processes involved, even if analogous to those proposed above for powdered tooth enamel and calcium phosphate, go on at a much slower rate.

Whole teeth were suspended in varying concentrations of unbuffered lactic acid at varying pH's. The amount of phosphate in the supernatants increased with increasing acidity. With the exception of 2 instances, no calcium was formed in the tubes to which fluoride had been added and in the 2 exceptions only a trace was found. The results are shown in Table V.

These results seem to support the mechanism proposed, viz. the tooth enamel is dissolved in the lactic acid and then the calcium reprecipitated, probably as calcium fluoride.

SUMMARY

Chemical studies on whole teeth, powdered enamel and calcium phosphate, suspended in lactic acid solution with and without added fluorides or oxalates, indicate that enamel is decalcified in lactic acid and the calcium redeposited as calcium fluoride.

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OBSERVATIONS ON INDUCED CARIES IN RATS[1]

V. Effects of Excessive Sugar in the Diet

F. J. McCLURE

From the Division of Physiology, Dental Research Section, National Institute of Health, Bethesda, Md.

The results of this study suggest that a synthetic diet containing an excessive quantity of sugar may under certain conditions induce dental caries in rats. Granulated sugar in rats' diets had a decided abrasive effect on the occlusal surface of molar teeth.

Attempts to produce caries in albino rats by excess quantities of sugar have generally resulted in failures (1, 2, 3, 4). Lilly and Grace (3) fed to 3 groups of 10 rats each, diets containing respectively 66% glucose, 66% lactose and 66% maltose. These rats failed to develop caries although fed 6½ months. Lilly (2) also obtained no obvious caries in 48 rats fed a 53% sucrose diet for 1 year. Rosebury and Karshan (1) found no caries by section diagnosis in 10 rats fed a 40% glucose diet over a period of nine weeks. Using a basal diet of Purina Mills Fox Chow, plus 55% candy (fudge), Day, Daggs, and Sedwick (4) found no caries among 40 rats on experiment 150–261 days. The fudge contained 78% "carbohydrate". The above experiments suggested the need for additional studies involving more rats fed diets containing larger quantities of sugar.

Prior to beginning the experiments reported here, we had available for dental examination, rats from nutrition studies in progress in the Division of Physiology.[2] Among 35 rats fed an adequate synthetic diet containing 73% glucose for a period of 180–270 days, 9 rats had large macroscopic cavities in 1 or 2 lower molar teeth. Another group of 20 rats received diets containing 65–72% cerelose for 142–150 days. Five of these rats showed cavities visible under low power magnification. These 55 rats were colony stock of the National Institute of Health. In contrast to the above rat-caries experiences, it may be noted that with this same colony stock 4 years ago, we failed to induce caries in rats fed diets containing 66% coarse ground dextrin. More recently we have found no caries in a group of 60 rats from this colony fed 1–1½ years, a diet containing 65.5% starch, 25.0% whole milk powder, 5.0% yeast, 1.0% cod liver oil and 0.5% salt.

EXPERIMENTAL

Rats 21 to 26 days old were selected by pairs, each pair being litter mates of the same sex. Diets were given *ad libitum* and carrots or kale were available

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[2] The author is indebted to Surgeon J. V. Lowry, USPHS and to Passed Assistant Surgeon A. Kornberg, USPHS for opportunity to examine the teeth of these experimental rats.

1 day a week. One rat of each pair received a control (starch) diet, the other a test (sugar) diet. The percentage composition of the diets follows:

Diet No.	526	526a	527	528
	%	%	%	%
Granulated sugar	70	—	—	—
Confectioners XXX sugar	—	70	—	—
Glucose (cerelose)	—	—	68.5	—
Corn starch	—	—	—	70
Casein	16	16	—	16
Whole milk powder	—	—	25.0	—
Osborne & Mendel salt mixture*	4	4	—	4
Wesson oil	3	3	—	3
Cod liver oil	2	2	1.0	2
Yeast	5	5	5.0	5
Sodium chloride	—	—	0.5	—

* Sodium fluoride omitted.

At the end of experimental periods, i.e., 200–270 days, rats were sacrificed and the teeth prepared for observation by autoclaving the head for 15 minutes to facilitate removing soft tissues. Caries was diagnosed microscopically and scored according to Cox, Dodds, Dixon and Matuschak (5). Molar teeth from representative non-carious rats in each group were extracted, dried and weighed in order to assess quantitatively an observed destructive action of granulated sugar on the occlusal surfaces.

Observation of caries experience by means of paired rats improves experimental control of litter and individual susceptibilities. Seasonal and environmental conditions such as temperature, humidity, light, handling, and confinement are also equalized. The final results are peculiarly susceptible to statistical treatment. There is no suspicion that variations in consumption of test vs control diets would affect differences between pair mates in the dental effects studied. Food intake was not equalized, therefore, as in the usual paired feeding method (6).

A significant outcome relative to caries experience occurred in experiment "A" (Table I) in which 10 rats of 27 pairs became carious when fed a sugar diet (diet 526). Dental caries under the conditions of this experiment is equally probable in each rat of a pair giving an ideal outcome of equal numbers of carious and non-carious rats on both control and test diets. In experiment "A" there was a deviation of 10 from the expected outcome. This is 3.8 times the standard deviation of 27 such trials[3] indicating with practical certainty that the outcome was not fortuitous.[4] This significant caries experience in rats was in complete agreement with our first observations regarding caries in rats fed cerelose in con-

[3] The standard deviation of the normal frequency distribution of the outcome of these 27 trials is given by the expression $\sqrt{0.5 \times 0.5 \times 27}$ which equals 2.6.

[4] According to Davenport's Table of Values of the Normal Probability Intergral Table IV, p. 166, Davenport and Eaks "Statistical Methods in Biology, Medicine and Physiology."

nection with nutrition studies in the Division of Physiology. These results alone suggest that under certain conditions excessive sugar in the white rats' diet may induce caries.

TABLE I

Caries in rats' molar teeth:—relation of excessive sugar vs. starch in rats' diets

Experiment	"A"		"B"		"C"		"D"		"E"	
Breeding stock	NIH		NIH		NIH		Sherman		Sherman	
Numbers of litters represented	15		8		12		12		5	
Number of paired rats	27	27	20	20	17	17	18	18	11	11
Diet No.	526	528	526a	528	527	527	526	528	526a	528
Principle component of diet	sucrose	starch	sucrose	starch	glucose	glucose	sucrose	starch	sucrose	starch
Average fluorine in diet (ppm)	3.4	4.2	2.8	4.2	2.0	2.0	2.5	4.2	2.8	4.2
Feeding period—days	250		200		270		250		365	
Caries diagnosis (according to Cox et al.—Ref. 9)										
Number of carious rats	10	0	1	0	1	0	0	0	2	0
Number of carious teeth	24	0	3	0	3	0	0	0	5	0
Number of carious areas	51	0	3	0	3	0	0	0	6	0
Total caries score	139	0	7	0	6	0	0	0	12	0
Fluorine content of sound molar teeth (1)										
	%	%	%	%	%	% (2)	%	%	%	%
Fluorine in upper molars	0.0057	0.0076	0.0071	0.0110	0.0110	0.2934	0.0085	0.0085	0.0085	0.0076
Fluorine in lower molars	0.0062	0.0054	0.0084	0.0094	0.0129	0.2972	0.0087	0.0072	0.0083	0.0090
Average fluorine all molars	0.0060	0.0065	0.0078	0.0104	0.0120	0.2953	0.0086	0.0079	0.0084	0.0083

(1) As shown in the table, no experimental diet contained more than 5.0 ppm. fluorine. This much fluorine ingested post-eruptively does not appear sufficient to increase the fluorine content of the molar teeth.

(2) These high values are due to 50 ppm. fluorine contained in the drinking water.

An examination of the exposed occlusal surfaces of these rats' molars indicated an abrasive effect of granulated sugar (Diet 526). The occlusal surface was hollowed out, leaving a crater-like appearance. The enamel being more resistant, formed a rim higher than the dentin but frequently was very irregular. This was in striking contrast with the fully retained, smooth occlusal surfaces of molars of starch-fed rats. On weighing the molar teeth of granulated sugar and

starch-fed rats, a statistically significant difference in weight of molar teeth was obtained (Table II). The question arose as to the possibility of dentin being affected by sugar in solution, per se, as well as being susceptible to an abrasive action of sugar granules. Seventy per cent of the granulated sugar fed in diet 526 would not pass a 60 mesh sieve, 25% would not pass a 40 mesh sieve.

Experiment "A" was repeated, therefore, using confectioner's powdered sugar all of which passed a 60 mesh sieve (Experiment "B"). There was no pro-

TABLE II

Loss in weight of rats' molar teeth due to abrasive action of granulated sugar (diet 526)

Experiment	"A"		"D"		"B"		"E"	
Breeding stock	NIH		Sherman		NIH		Sherman	
Number of pairs of rats	16		10		16		10	
Diet	526	528	526	528	526a	528	526a	528
Diet, principle component	sugar	starch	sugar	starch	sugar(1)	starch	sugar(1)	starch
Days on experiment	250		250		200		265	
Final empty weight (gm.)	252	225	204	201	190	178	211	208
Weight of molar teeth								
Right lower (mg)	29.2	31.9	29.9	31.8	29.5	29.7	36.5	37.6
Difference (2) (mg)	−2.7		−1.9		−0.2		−1.1	
Probability (3)		<0.01		>0.05		0.8		0.2
Significant	yes		almost		no		no	
Right upper (mg)	28.3	31.6	29.2	31.2	29.2	29.1	34.6	35.9
Difference (2) (mg)	−3.3		−2.0		+0.1		−1.3	
Probability (3)		<0.01		<0.05		0.8		0.2
Significant	yes		yes		no		no	
All right molars (mg)	57.5	63.5	59.1	63.0	58.7	58.8	71.1	73.5
Difference (2) (mg)	−6.0		−3.9		−0.1		−2.4	
Probability (3)		<0.01		<0.05		0.8		0.4
Significant	yes		yes		no		no	

(1) Confectioners powdered sugar.

(2) "Sugar-rat" molars differ from "starch-rat" molars.

(3) Table of Values of t. R. A. Fisher and F. Yates. Statistical Tables for Biological, Agricultural, and Medical Research.

nounced effect detected from this sugar diet (Diet 526a) on these rats' molars. Differences in weights of teeth of rats fed the powdered sugar diet vs. rats fed starch diet 528, were not statistically significant (Table II).

In Experiment "B", only 1 sugar-fed rat in the 20 pairs developed caries, a truly different outcome from Experiment "A". Experiments "D" and "E" were started then, using a Sherman strain of rats obtained from the Rockland Farms colony, New City, N. Y. These 2 experiments again showed an abrasive effect of diet 526 in contrast to diets 526a and 528. The effect is shown by

statistically significant differences in weight of molars in pairs of rats in Experiment "D", but not in paired rats in Experiment "E" (Table II). Only 2 rats of this group, among 29 rats receiving the sugar diets, developed caries. None of the 29 starch pair-mates developed caries.

Experiment "C" was planned in the hope that glucose in diet 527 might be more effective in inducing caries than sucrose in diets 526 and 526a. Both rats of a pair in Experiment "C" received Diet 527. One rat of each pair, however, was given drinking water containing 50 ppm. fluorine as sodium fluoride. It was anticipated that if these rats proved susceptible to caries it would be possible to ascertain what effect fluorine might have on this type of caries. The results, as will be noted, were not as expected. Only one of these 34 rats receiving this glucose diet had microscopic caries.

Fluorine was determined in whole sound molar teeth according to group and diet association (Table I). Those rats in experiment "C" which received 50 ppm. fluorine in their drinking water markedly increased the fluorine content of their molar teeth post-eruptively. This was to be expected (7, 8). Data for fluorine in molars of rats in experiment "A" do not indicate striking differences from other groups (aside from the exceptional rats just noted above). Although the fluorine figures of experiment "A" are lowest for all groups, they do not appear sufficiently different by comparison to account entirely for the unusually high incidence of caries experienced in experiment "A".

Prenatal and lactation exposure to fluorine, nevertheless, may be one of several factors which influenced the caries response of these rats. There is no reason to believe that fluorine in the experimental diets (Table I) influenced the caries outcome.

DISCUSSION

The significant rat caries experience which developed in Experiment "A", as well as the unusual caries incidence which was noted in observations made preliminary to these studies, seems highly suggestive of a rôle of sugar in rat caries production. It seems notable that among a total of 110 rats receiving sugar diets, 14 rats (12.6%) developed microscopic caries, whereas not one of 76 rats fed a starch diet ever showed microscopic caries. The positive caries results of this study so far as is known are the first to be reported in connection with excessive sugar in rats' diets.

Failure to reproduce in Experiments "B" and "C" the unusual rat caries experience of Experiment "A" has been attributed to a change in caries susceptibility of rats produced in the colony at the National Institute of Health. These rat-caries studies extended over a period of approximately 3 years, starting in September 1942. During this time rats obtained at the National Institute of Health improved notably as regards mortality, weight at weaning age, vitality, and general appearance. Also a "home-made" stock diet was changed to Purina Chow pellets. Failure to produce caries in Sherman strain rats (Experiment "D" and "E"), also suggests that we were dealing with a highly caries-susceptible group of rats in our early experiments.

Fluorine in mothers' diets and in diets given prior to and after tooth eruption, affects the fluorine content of dentin and enamel and appears responsible for a reduction in rats' dental caries experience as induced by coarse corn meal diets (9, 7, 8). Purina Chow stock diet which we have found may contain as much as 15 to 20 ppm. fluorine was not being fed the rat colony at the National Institute of Health at the time of experiment "A". Reduced caries susceptibility, therefore, as shown in Experiments "B" and "C" and in experiments with rats of the Sherman strain (Experiments "D" and "E") may possibly be related to a protective effect of fluorine acquired by the rats' teeth during growth and calcification. Although inconclusive, the fluorine data emphasize strongly the importance of controlling the fluorine in rations fed to colony stock if any attempt is made to stabilize caries susceptibility in the offspring.

Our finding an abrasive effect of granulated sugar on rats' molar teeth is of interest since Shaw, Schweigert, Elvehjem and Phillips (10) also report unusual abrasion in cotton rats fed sucrose diets as well as other diets. Caries artefacts have been suggested as arising from fractures induced by coarse particle corn and rice diets in albino rat studies. In the case of the cotton rat the experimental period is kept at 14 weeks because fractures appear to produce caries artefacts (10). The evidence of an abrasive action of granulated sugar particles on rats' molar tooth surfaces emphasizes the significance of particle size in all diets suggested for experimental caries studies. A particle of sufficient hardness and size to cause abrasion may contribute to the fracture of undermined carious cusp areas.

Value of the albino rat for caries studies has remained questionable because this animal presumably must be fed a coarse-particle diet to induce caries. Susceptibility varies widely among litters (11) and among individuals. Solution of rat caries problems will be furthered, therefore, by preparation of synthetic caries-inducing diets of powder-consistency, by evaluation of the rôle of diets fed during prenatal life and during lactation, and mainly by development of strains of animals which are consistently caries susceptible.

In a notable recent work, Hunt, Hoppert and Erwin (12) demonstrated inheritance factors involved in rat caries-susceptibility. "For the first time, no doubt, in the history of dental caries research, there is a uniform strain of rats known to be highly susceptible to dental caries when fed a specific diet" (12). Although the "specific diet" was a coarse particle rice diet, the results suggested similar inheritance factors relative to a powdered diet. The significant caries produced in our first group of rats, followed by failure in other groups, emphasizes the importance of stabilizing inherent susceptibility in animals selected for the purpose of studying experimental caries.

SUMMARY

Contrary to reports of similar studies, groups of white rats developed a significant incidence of microscopic dental caries when fed synthetic diets containing excessive quantities of sucrose and glucose. Control rats fed a similar diet in which starch replaced the sugars developed no caries. Failure to duplicate

initial success with excessive sugar diets is attributed to a reduced caries susceptibility in animals available for subsequent studies. There was some evidence that fluorine exposure during formative tooth life may have been one of several unknown and uncontrolled pre-experimental factors which caused the erratic caries response among the several groups of rats. A diet containing 70% granulated sugar had an unmistakable abrasive action which was noted on the occlusal surfaces and particularly in the exposed dentin of rats molar teeth.

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STUDIES ON THE OXIDATION-REDUCTION POTENTIALS OF SALIVA[1]

L. L. EISENBRANDT, PH.D.

From the Research Laboratory, School of Dentistry, University of Kansas City, Kansas City, Mo.

The literature pertaining to the oxidation-reduction potentials of saliva is limited, possibly because of the complexity of the subject. Pincus (1) was the first to recognize oxidation-reduction phemonena in saliva when he noticed its marked reducing properties. Both methylene blue and dichlorphenol-indophenol became colorless within a few seconds after contact with saliva. Appleton (2) made observations similar to those of Pincus and, further, that reducing properties varied with the individual. Apparently Wessinger (3) was the earliest to report the use of electrometric methods for determining these potentials of saliva. Since a stable end point seemed impossible, he concluded that no accurate data of this nature were obtainable for saliva. Eisenbrandt (4) reported that initial oxidation-reduction potentials, determined immediately after the collection of a salivary sample, did not vary with the subject nor with time of collection when based upon bi-hourly intervals during the day. These results were in marked contrast with the pH of the same salivary samples; the pH varied with the subjects and with the time of collection.

The data herein presented are a continuation of the earlier study with the inclusion of additional factors which are related to the E_h of saliva. No attempt was made to remove bacteria or epithelial cells, rather it was desired to test the normal saliva immediately after removal from the mouth; neither was an attempt made to obtain final potentials of saliva.

METHODS

The procedure, subjects, time and expressions of significance were identical to those in an earlier paper on the pH of saliva (5). Approximately 5 ml. of unstimulated saliva were collected in the mouth and then deposited into a sample cup. The E_h of saliva was determined with a gold-platinum electrode and almost simultaneously determinations of the pH were made with the glass electrode. Emphasis is placed upon the fact that the readings were obtained within 1 minute after removal of the sample from the mouth and that during this brief time interval the E_h did not change. The temperature corrections for E_h were made immediately after testing each sample.

Of the 7 subjects available for the experiment, 4 were tested throughout the year, 1 for 10 months, 1 for 6 months and 1 for 4 months. Five tests were made at bi-hourly intervals from 9 A.M. to 5 P.M. during the day for 1 week each month for 12 months. The statistical methods used applied for interpre-

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TABLE I

Analysis of monthly variations in the E_h of saliva

	OCT.	NOV.	DEC.	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	TOTAL
No. readings	135	117	83	115	129	149	118	119	145	149	148	148	1555
Mean E_h (mv.)	+270	+248	+263	+266	+289	+288	+274	+282	+293	+336	+362	+394	+301
Std. error of mean	∓7.17	∓2.87	∓3.17	∓2.43	∓1.99	∓2.42	∓2.14	∓1.82	∓2.79	∓3.23	∓3.74	∓2.94	∓1.83
Total mean ratio	4.31*	18.27*	11.87*	14.58*	6.00*	5.42*	12.85*	16.11*	2.86*	10.94*	16.49*	32.07*	

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES
Total	1,554	4,370,020	
Between monthly group means	11	2,930,777	266,434
Readings within months	1,543	1,439,243	933

F = 266,434/933 = 285.57.....highly significant

1% Significance = 2.26

* = Significantly different from +301 mv.

tation were the same as those used previously (5). Some of the tables are abbreviated and the following key is employed to designate significance: * denotes significance, † denotes probable significance, — denotes no significance. The terms "mean" and "average" are used synonymously.

EXPERIMENTAL DATA

For 1 week each month during 12 months oxidation-reduction potentials of saliva of 7 subjects were determined; a total of 1555 readings were recorded. The mean E_h was +301 mv. and the standard error of the mean was 1.83. The range of single readings was from +158 to +542 mv.

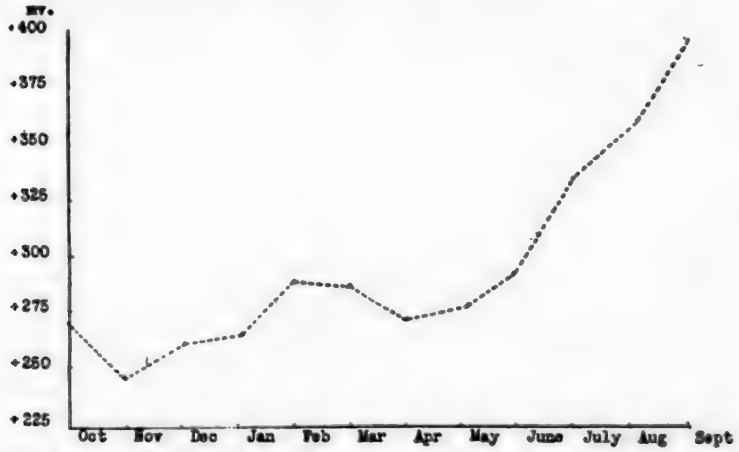

FIG. 1. Monthly averages of E_h of saliva for the group. Subsequent monthly tests following termination of experiment showed that E_h peak was in September and by December E_h had dropped 100 mv.

Monthly Variations of the Group

The monthly mean E_h of saliva for the subjects, considered as a group, was recorded and an analysis of variance determined; the results are summarized in Table I. The lowest mean E_h was +248 mv. obtained in November; the highest was +394 mv. in September. The variance during the year at monthly intervals was highly significant. *Fig. 1* shows the striking changes from month to month and the general increase during the year. The remarkable increase in E_h from May until September was unexpected and still unexplained. Following the termination of the experiment, additional tests were made in November upon 4 of the subjects; the average E_h was +332 mv., indicating a considerable decrease from the high of +394 mv. in September. A few additional tests during the following month of December indicated a still further decrease in E_h (average +294 mv.). These extra tests were helpful in showing that September marked the end of the outstanding rise in E_h during the late summer of the particular year studied.

Table I also includes the total mean ratios which indicate that the E_h of saliva at each month was significantly different from the annual mean of +301 mv. The E_h of saliva in June (+293 mv.) was the nearest to the annual mean. The greatest positive deviation in E_h from the year's average was +93 mv. in September, and the greatest negative deviation was +53 mv. in November. From the beginning of the experiment in October through June (9 months) the monthly mean E_h of saliva was below the year's average (+301 mv.) and only during the remaining 3 months (July, August and September) were the mean E_h values of saliva above the annual mean. An explanation is not available at present for the fact that the E_h of human saliva tends to vary with the seasons of the year. It is likely that this phenomenon is correlated with many other physiological activities which change with the seasons. The pH of saliva as reported (5) varies significantly with the seasons, months, bi-hourly periods of the day and with the individuals.

TABLE II

Analysis of variance on the E_h of saliva of 7 subjects

	H	S	C	M	P	E	R	GROUP
No. readings	140	268	258	231	283	275	100	1555
Mean E_h (mv.)	+274	+300	+301	+308	+295	+300	+346	+301

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES
Total	1554	4,370,020	
Between subjects	6	330,535	55,089
Between readings of each subject	1548	4,039,485	2,609

F = 55,089/2,609 = 21.11.....highly significant
1% Significance = 2.82

INDIVIDUAL VARIATIONS

Table II summarizes the annual mean E_h of saliva of each subject. The 2 women, H and R, had the extreme averages which are not attributed to sex but to the particular time of year when each was in the experiment. The saliva of H was tested 140 times and averaged +274 mv., which was the lowest mean obtained; R's saliva, tested 100 times, averaged +346 mv. and was the highest mean for any individual. The remaining 5 subjects, all males, were nearly identical as to the E_h of saliva; their averages were between +295 and +308 mv. (*fig. 2*). Approximately 250 tests were made upon each male. An analysis of variance of the subjects was highly significant, showing that they differed from one another.

The significance of the statistics was dependent upon the subjects H and R. The former was tested from October through March and the latter from June through September. Had H and R been available throughout the year, their averages probably would have been much like that of the others. *Fig. 1* shows

the relatively low E_h in the winter when H was available and the high E_h during the summer when R was in the experiment. Interestingly enough, deletion of data of H and R altered the annual mean only 1 mv. (i.e. from +301 mv. to +300 mv.). Also, when data from October through March were studied with or without H, the means were the same. Likewise, the data from June through September had the same mean with or without R.

The data for the entire year were studied statistically after excluding the readings of H and R. The F ratio was 2.06, whereas the ratio required before the data could be considered significant was 3.34. Hence, the subjects were similar in E_h characteristics. This contention is further strengthened by M whose saliva was tested 10 months. M entered the experiment after the 2 lowest

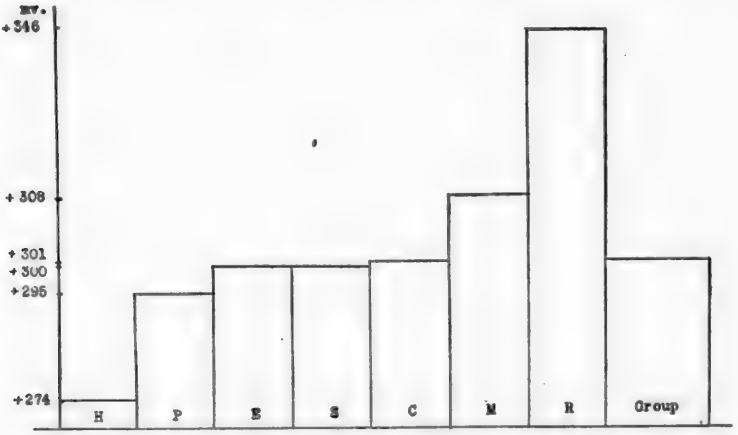

FIG. 2. The annual mean E_h of saliva for each subject and for the group.

monthly periods were passed (October and November). His saliva tended toward the same characteristics as R who was tested only during the last phase of the experiment. Therefore, on the basis just discussed, there is justification in stating that subjects do not vary from one another as to the E_h of saliva when the tests are made at comparable periods of the year.

The E_h of subjects' salivas was compared on a monthly basis. The data are illustrated in *fig. 3* which clearly shows the marked similarity in salivary E_h of each subject at each month. The E_h in the fall months is low for each subject and during the late summer is high. This figure also aids in clarifying the preceding paragraph; the blocks for H and R resemble those of the other subjects for like periods. Statistically, each subject was significantly variable from month to month. Even H and R varied sufficiently to be significant in the E_h of their saliva from month to month during the time they were in the experiment.

The salivary E_h of the subjects was compared at each month. The E_h was not statistically variable except during July. For the other 11 months the salivary E_h of the subjects was remarkably similar. These data suggest that temporary oxidation-reduction potentials of saliva are not a function of individual variations, but of time on a monthly or perhaps a seasonal basis.

Bi-hourly Variations

As with the pH, the E_h of saliva of the group was considered on a bi-hourly basis during the day. The group means at each period (9 and 11 A.M. and 1, 3 and 5 P.M.) were not significantly variable (Table III); the range of the annual means was from +298 mv. at 5 P.M. to +304 mv. at 9 A.M. *Fig. 4* is interesting when compared with the same figure on the pH of the same salivary samples (5). The 2 figures were reciprocal i.e.,E_h was highest at 9 A.M. when

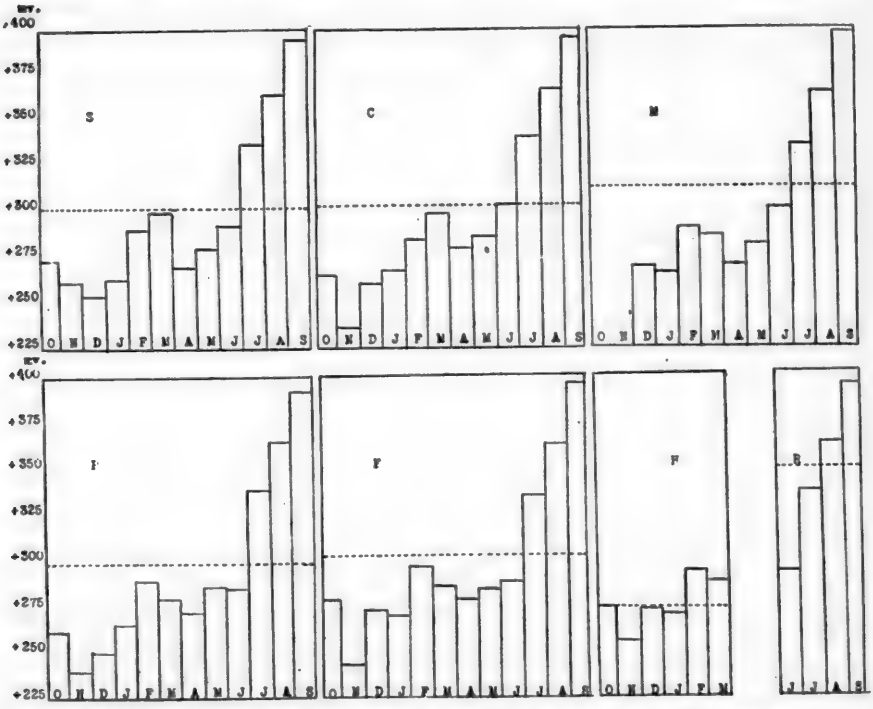

FIG. 3. Monthly mean for each subject; dotted line in each graph denotes subject's average.

the pH was lowest (+304 mv. and pH 6.55) and both passed through their respective means at 1 P.M. At 5 P.M. the E_h was low and the pH was high (+298 mv. and pH 6.71). Although the figures were reciprocal, the pH of saliva was significantly variable and the E_h was not. In summary, the E_h of saliva of a group of individuals apparently was not a function of bi-hourly periods during the day and neither did the E_h vary appreciably during the day.

Fig. 5 compares each bi-hourly period from month to month for mean variations in the temporary oxidation-reduction potentials of saliva for the group. At 9 A.M. the monthly mean E_h ranged from +250 mv. in November to +397 mv. in September. Likewise, the other bi-hourly periods were highly significant

in variability from month to month. In June sharp increases in E_h began for each period. *Fig. 5* also shows the increase in the E_h of saliva during the year and the marked similarity of the 5 bi-hourly periods at any 1 month. Although the patterns of the bi-hourly curves were similar, especially in the spring months, there were significant variations in December, February and August.

TABLE III

Bi-hourly variations in the E_h of saliva for 1 year

	9 A.M.	11 A.M.	1 P.M.	3 P.M.	5 P.M.	TOTAL
No. readings	310	316	312	311	306	1555
Mean E_h (mv.)	+304	+298	+302	+304	+298	+301

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES
Total	1554	4,370,020	
Between bi-hourly periods	4	11,220	2,805
Between readings in each period	1550	4,358,800	2,812

F = 2,812/2,805 = 1.00.....not significant
1% Significance = 13.48

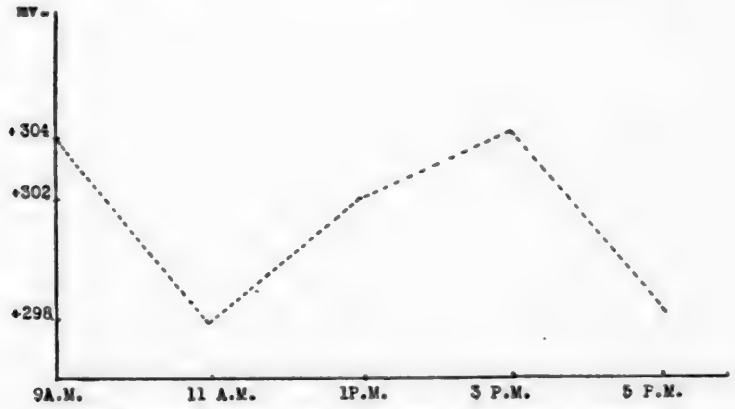

FIG. 4. Mean E_h of saliva for the group at bi-hourly intervals.

A comparison was made of the E_h of subjects' salivas at each bi-hourly period. For all 5 periods the subjects had E_h values of saliva which were significantly variable from each other (Table IV). However, the latter condition was due to H and R; the other subjects were much alike (*fig. 6*). Removing H and R from the expermental data because of incomplete records renders the variations in the E_h of saliva insignificant. Therefore, when tested at comparable periods, the subjects are not significantly variable with each other at any 1 period of the day. The saliva of each subject was not variable during the day at bi-hourly intervals (read down in Table IV). The evidence seems to indicate that the

temporary oxidation-reduction potentials of saliva were more influenced by the time of year than by individual variations.

A summary was made of the E_h of saliva of each individual at a particular bi-hourly period of each month of the year. The E_h for all subjects except H and R were significantly variable at each time period throughout the year which is evidence that the saliva varied according to months at any 1 bi-hourly period. R's saliva was not variable at 9 A.M. and the E_h of H's saliva had little variability at any period unless possibly at 11 A.M.

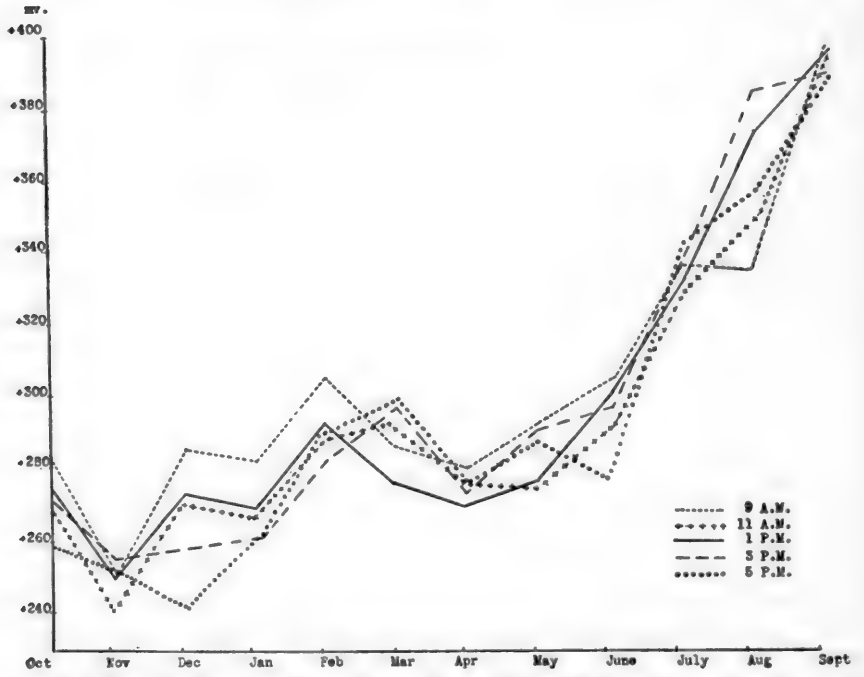

FIG. 5. How E_h of saliva varies at each bi-hourly period during 1 year, and how the 5 periods vary from one another at each month.

The exceptions to the E_h trends in saliva used in this experiment seemed due to H and R. Their variations suggest that less fluctuations in the E_h occurred in the fall and winter than in the summer. Consequently, H had little variation and R had considerable variation due to the greater changes which occurred during the summer. Presumedly their data were representative of the other subjects had they been analyzed for those particular months i.e., summer months for all or winter months for all.

DISCUSSION

As saliva is a composite biological fluid constantly changing in its bacterial, chemical and cellular make-up physiological standards are somewhat difficult

TABLE IV

E_h of saliva studied over period of 1 year showing how subjects varied from one another at specific times of day; also, how each subject varied during bi-hourly intervals

	S	C	P	M	E	H	R	ANALYSIS OF VARIANCE
(9 A.M.)								F 2.88
No. readings	54	52	53	47	56	28	20	1% Sign 2.85
Mean E_h (mv.)	+302	+304	+300	+311	+305	+278	+338	Sign *
(11 A.M.)								F 5.13
No. readings	54	54	58	46	56	28	20	1% Sign 2.85
Mean E_h (mv.)	+300	+298	+294	+301	+296	+269	+345	Sign *
(1 P.M.)								F 6.41
No. readings	55	49	58	46	56	28	20	1% Sign 2.85
Mean E_h (mv.)	+299	+302	+291	+310	+300	+273	+360	Sign *
(3 P.M.)								F 4.16
No. readings	54	51	57	47	54	28	20	1% Sign 2.85
Mean E_h (mv.)	+305	+306	+296	+314	+299	+274	+347	Sign *
(5 P.M.)								F 3.46
No. readings	51	52	57	45	53	28	20	1% Sign 2.90
Mean E_h (mv.)	+293	+297	+294	+305	+302	+274	+340	Sign *
Analysis of variance								
F	2.56	2.05	4.17	2.50	4.10	3.14	2.24	
1% Sign	13.52	13.52	13.52	13.52	13.52	13.57	13.57	
Significance	—	—	—	—	—	—	—	

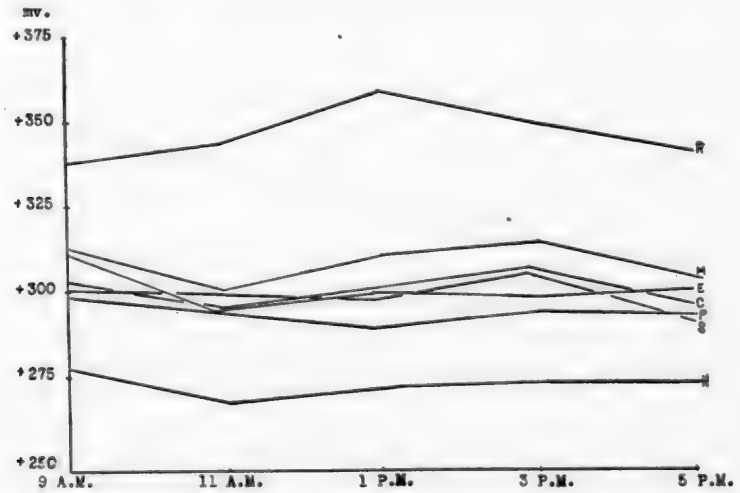

FIG. 6. Variations in E_h of saliva at bi-hourly intervals for each subject, also, between subjects for each time interval. Repeated tests were made for 1 year.

to determine. By carefully controlling the method and time of collecting saliva, and by making corrections for the temperature of saliva, reasonably reproducible E_h data were obtainable; such data were classed as temporary because the saliva was tested immediately after removal from the mouth. The E_h under these conditions was approximately the same as that tested by inserting the electrodes directly into the mouth. However, if saliva were kept in the sample cup beyond 2 minutes a considerable but irregular drift toward a negative potential began. After 24 hours the E_h was highly variable and usually negative. It was observed that these drifts occurred whether the sample was exposed to air or sealed with paraffin.

Especially noteworthy were the significant variations during a period of 12 months in the E_h of saliva of the group and of each individual within the group. Also, there were considerable fluctuations each month from the monthly mean. Particularly outstanding was the fact that the individuals were not significantly variable with one another in E_h characteristics of saliva. In other words the E_h of saliva seems to be influenced more by time (monthly) than by individuals. If the saliva is analyzed on the basis of bi-hourly variations during the working period of the day, the E_h does not change either for the group or for the individual. The evidence indicates that seasons may influence the E_h of saliva. Seasons apparently do affect other phenomena of the mouth. Miller and Greenhut (6) stated that they found seasonal changes in the rate of necrotic gingivitis; the disease is more prevalent in the fall (October) and least in the summer (August). The caries susceptibility rate appears to vary with the seasons (7); the index is 2 times as great in the winter as in the summer. The pH of saliva (5) seems to vary with the seasons.

The advisibility of deleting the data of H and R was considered since they were not in the experiment for the full period. It was believed desirable to use their data because their saliva closely resembled that of the other subjects at the specific months each was in the experiment (*fig. 3*) and actually strengthened the contention that time or season causes variations in the E_h of saliva. In addition to the statistical data in Table II and Table III, which show significant variations in subjects for the year and at a particular time of day, respectively, data of H and R were deleted and analysis determined. In neither case was the variation significant. Furthermore, the inclusion of their data did not alter the monthly means, and the annual mean changed only 1 millivolt.

SUMMARY

(1) The average initial oxidation-reduction potential (E_h) of resting saliva was +301 mv. for 7 subjects tested electrometrically 1555 times. The pH was 6.64. (2) The E_h of saliva of the group of subjects changed markedly over a period of 12 months. (3) The group's mean E_h of saliva for each month was significantly different from the annual mean of +301 mv. (4) Subjects did not vary from one another in the E_h of saliva when tested under identical conditions. (5) At each month the subjects' salivas were remarkably similar in E_h. (6) The E_h of saliva of a subject was quite variable from month to month for 1 year as

tested in this experiment. (7) The E_h of saliva for the group did not fluctuate to any extent during the day. (8) Marked fluctuations in the E_h of saliva for the group occurred from month to month at each of the 5 bi-hourly periods. (9) Subjects were not much different from one another at any 1 of the bi-hourly periods of the day. (10) The E_h of saliva of a subject at a particular time of day varied greatly from month to month.

CONCLUSION

The temporary oxidation-reduction potentials (E_h) of saliva, as tested in this experiment, appear to be a function of time based upon monthly periods or seasons but not upon bi-hourly periods during the day. Apparently the E_h of saliva is not a function of the individual.

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EVALUATION OF ANTIMICROBIAL AGENTS. THE GERMICIDAL ACTIVITY OF A SYNTHETIC DETERGENT, DC-12[1]

M. E. PIERCE AND E. B. TILDEN

Department of Bacteriology, Northwestern University Dental School, Chicago, Ill.

Dependence upon chemical methods for the destruction of microorganisms is regarded by the bacteriologist as justifiable only when the clean and certain method of sterilization by heat is impossible. No available chemical disinfectant is able in a reasonable time to accomplish what the autoclave so quickly and surely accomplishes, namely, the destruction of the spores of bacteria and fungi and of the organism of tuberculosis. Phenol, while effective against the 2 latter organisms (1, 2), is ineffective against bacterial spores (3). This limitation, indeed, is shared by most disinfectants, which often require a period of hours or even days to destroy spores. Saponated cresol (4), which is probably the most efficient disinfectant available at present, is used widely in hospitals and bacteriological laboratories but has not found favor with the dentists because of its odor. The compounds of mercury are now known to be bacteriostatic rather than bactericidal (4), and when they are washed away leave the bacteria alive and ready to multiply. Formalin (4), a time honored disinfectant, has recently been recommended for dental instruments by Tainter, Throndson, Beard and Wheatlake (5), who have found a means of disguising the penetrating odor. Phenol and cresol are perhaps the only disinfectants which are recognized as being active against the organism of tuberculosis.

The standard test of the efficiency of a substance proposed as a disinfectant is still the comparison of its germicidal activity *in vitro* with that of phenol. The limitations of this test have become more and more evident in recent years, and it is well known that bacteriostatic substances such as mercurials may have high phenol coefficients. The present trend among research workers is toward practical tests which simulate actual conditions. We have chosen 2 such practical tests and have examined by these procedures such substances as are applicable to the disinfection of instruments, dental or surgical, for which heat sterilization is not practicable (6, 7).

The present report is chiefly concerned with the evaluation of a quaternary ammonium salt, di methyl benzyl lauryl ammonium chloride.[2] The germicidal activity of compounds of this type is presumed to be in the fatty acid (in this case the C-12) group. We shall refer to the substance as DC-12. The crystals are extremely soluble in water. The solution is colorless and has a slight aro-

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[2] Prepared by the Stillman Company, Aurora, Illinois.

matic odor. It is no more corrosive to instruments than is distilled water. Sodium nitrite, added in a concentration of 0.2%, prevents rusting.[3]

DETERMINATION OF PHENOL COEFFICIENT

Determination of phenol coefficients were made by the standard F. D. A. procedure (8), without organic matter (Table I) and in the presence of 10% serum (Table II). The phenol coefficient was 400–500 in the absence of organic matter. When the test was done in the presence of 10% serum, the coefficient was reduced, but dilutions of 1:5,000 were still germicidal.

TABLE I

Phenol coefficients. Tests without added organic matter

BACTERIUM	TEMP.	DILUTION KILLING IN TEN MIN. BUT NOT IN FIVE MIN.		PHENOL COEFFICIENT
		Detergent	Phenol	
*S. aureus**	20°	1:40,000	1:70	570
*S. aureus**	20°	1:30,000	1:70	429
*S. aureus**	20°	1:35,000	1:65	538
*S. aureus**	37°	1:40,000	1:80	500
*S. aureus**	37°	1:50,000	1:85	588
*S. aureus**	37°	1:50,000	1:90	555
*S. aureus***	37°	1:40,000	1:90	444
B. typhosum†	20°	1:15,000	1:100	150
B. typhosum†	20°	1:25,000	1:100	250
B. typhosum††	20°	1:30,000	1:85	353
B. typhosum††	37°	1:55,000	1:120	459
B. typhosum††	37°	1:55,000	1:120	459

* Culture of *S. aureus* recently isolated from a boil (Culture W.T.) was used.

** F.D.A. 209, obtained through the courtesy of Dr. C. A. Colwell, Valsicol Corp., Chicago.

† Culture from collection in Northwestern University Medical School, obtained through the courtesy of Dr. A. W. Walker.

†† Culture obtained through the courtesy of Dr. Glenn Slocum, Food and Drug Administration.

Solutions of DC-12 in 1:1000 concentration showed no decrease in their phenol coefficients after being kept for a period of 9 months.

Like other detergents (9), DC-12 is incompatible with soap, the coefficient being only 2.5 in the presence of 0.4% Ivory soap flakes.

Tests for sporicidal activity

Cultures of spore-forming bacteria were selected which produce a relatively homogeneous growth in broth (*Bacillus subtilis*, *Bacillus cereus*).[4] After an incu-

[3] No rusting occurred even when instruments had been left for 10 days in the solution of DC-12 containing sodium nitrite. The latter does not interfere with germicidal activity.

[4] *Bacillus cereus* 201 and *Bacillus subtilis* 261. We are indebted for these cultures to Dr. Nathan R. Smith, of the United States Department of Agriculture.

bation period of 48 hours, the culture was shaken well and the coarse particles allowed to settle. The supernatant was transferred by pipette into another tube and heated at 80°C. for 15 minutes. Serial dilutions were plated; the number of colonies obtained in 48 hours was 200,000 in each ml. Five tenths ml. were used in the test, which was carried out from this point according to the standard procedure.

DC-12 was found to be effective against both *B. subtilis* and *B. cereus* in 10 minutes in a 0.1% solution. Phenol did not destroy the spores of *B. subtilis* even when used in a saturated solution, but a 5% solution was able to kill the spores of *B. cereus* in 10 minutes.

Test for fungicidal activity

As a fungicide, DC-12 was disappointing. We followed the procedure recently proposed by Emmons and others,[5] using a spore suspension of *Trichophyton*

TABLE II

Phenol coefficients. Tests made in presence of organic matter (10% rabbit serum)

BACTERIUM*	TEMP.	DILUTION KILLING IN TEN MIN. BUT NOT IN FIVE MIN.		PHENOL COEFFICIENT
		Detergent	Phenol	
S. aureus	20°	1:8,000	1:65	123
S. aureus	20°	1:10,000	1:80	125
S. aureus	37°	1:15,000	1:90	167
S. aureus	37°	1:20,000	1:100	200
B. typhosum	20°	1:6,000	1:95	63
B. typhosum	20°	1:5,000	1:90	56

* The standard (F.D.A.) cultures were used throughout this experiment.

mentagrophytes, with phenol as a control. DC-12 showed no fungicidal activity even in 5% concentration.

RUBBER RING TEST

Nungester and Kempf (6) devised, for class use, a procedure for testing disinfectants *in vitro* which gives indications of both bacteriostatic and bactercidal action. Several small rubber rings are contaminated with a culture, one for each of the time intervals during which it is desired to have the disinfectant act. After exposure to the solution for the desired length of time, a rubber ring is rinsed briefly in broth to remove the disinfectant and then deposited in a tube of broth, which is incubated for 48 hours.

Various bacteria differ greatly in their resistance to disinfectants, hence it would be a great advantage to have a test in which we are not limited to those which grow on plain broth, as we are in the standard test. Accordingly, we sub-

[5] Emmons, C. W., Proposed method for testing fungicides against Trichophyton. *Am. J. Publ. Health*, **35**: 844, 1945. See also Weidman, F. D., Emmons, C. W., Hopkins, J., and Lewis, G. M., War and Dermatophytosis, *J. A. Med. A.*, **128**, 805–11, 1945.

stituted in the rubber ring test (except for the rinsing), a richer medium, Difco brain heart infusion containing 1 part in 10 of serum (rabbit) and made semisolid with 0.15–0.2% agar, which is suitable for the growth of a great variety of bacteria. The conditions in the oral cavity are simulated by the viscosity of the medium and the content of organic matter. The broth is used for the rinsing, as in the original procedure.

Several pyogenic cocci were examined by the modified rubber ring test, with the results shown in *Table III*. *Diplococcus pneumoniae* was the least resistant to the action of DC-12, and *Staphylococcus aureus* was the most resistant. The streptococcus of the *viridans*, or alpha group, was more resistant to the action of DC-12 (and phenol) than the beta streptococcus. DC-12 proved effective in 0.1–0.2% solution under the conditions of this test.

TABLE III

Germicidal tests by the rubber ring technique

	TIME REQUIRED TO KILL						
	DC-12				Phenol		
	1:500	1:1000	1:5000	1:10000	1:20	1:40	1:80
D. pneumoniae (Type 1).		1 min.	1 min.	5 min.		1 min.	5 min.
Streptococcus viridans (alpha).....	5 min.	5 min.	15 min.		5 min.	15 min.	
Streptococcus pyogenes (beta) (Type C).....	1 min.	1 min.	5 min.		1 min.	5 min.	5 min.
Staphylococcus aureus...	1 min.	15 min.			1 min.	5 min.	15 min.

As respresentative of the mercurials, metaphen was examined by the rubber ring test. Its germicidal activity was low under the rather rigorous conditions of this test. The phenol coefficient of the solution used[6] was 62.5.

MOUSE TEST

A simple technique for evaluating disinfectants *in vivo* was devised by Nungester and Kempf (7), who call it the "infection-prevention test." A procedure of this sort has obvious advantages over one carried out in the test tube. The detection of a few bacteria which survive the action of a disinfectant is far more likely when they are injected into the susceptible animal body than when deposited in the culture tube. This fact was definitely proved by Nungester and Kempf with regard to the pneumococcus (7). Moreover, any disinfectant introduced with the culture is rapidly diluted and washed away by the body fluids, so that the possibility of bacteriostatic action is reduced and perhaps even nullified. A substance which is effective by this sort of test is likely, therefore, to be

[6] A solution of metaphen in 1:500 concentration, obtained in the open market, was used in all the tests reported in this paper. We have since obtained, through the courtesy of the Abbott Laboratories, solutions which contain 4% of benzyl alcohol. Our examinations of these solutions will be reported later.

an efficient germicide. The American Medical Association had advised the use of the procedure (10) in the evaluation of any new disinfectant proposed.

The procedure is as follows: Mice are anesthetized and the tail swabbed with a culture highly pathogenic for the mouse. (Nungester and Kempf used the pneumococcus and a hemolytic streptococcus; we have used the pneumococcus as the test organism.) The contaminated tail is exposed to the disinfectant for 2 minutes, then the tip (1 cm.) is cut off and implanted aseptically in the peritoneal cavity. Survival of the animals shows that the disinfectant has killed most, if not all, of the organisms, while a high mortality, with recovery of the organism from the heart blood of the animal, indicates failure of the disinfectant to kill all of the organisms. As a control for the virulence of the culture, physiological salt solution is used in place of the disinfectant for one group of mice in each experiment.

TABLE IV

Germicidal tests by rubber ring technique

	TIME REQUIRED TO KILL					
	Metaphen*			Phenol		
	1:500	1:1000	1:2500	1:20	1:40	1:80
D. pneumoniae (Type 1)	5 min.	10 min.	15 min.		5 min.	5 min.
Streptococcus viridans (alpha)	Not killed in any dilution tested			5 min.	10 min.	
Streptococcus pyogenes (beta, Type C)	Not killed in any dilution tested			1 min.	1 min.	
Staphylococcus aureus		5 min.	Not killed	5 min.	5 min.	15 min.

* Except in the 1:2500 dilution, the broth tubes in which the ring was rinsed showed no growth, i.e., the 1:500 and 1:1000 dilutions were bacteriostatic but not bactericidal.

Nungester and Kempf tested certain mercurials and one of the cationic detergents, using aqueous solutions in one series and tinctures in another. Two other series of experiments were carried out, one with 70% alcohol, and one with 50% alcohol containing 10% acetone. These were added because the mortality when tinctures were used was less than with aqueous solutions. The record of their results is reproduced in Table IV, which shows the inefficiency of the mercurials and alcohol[7] and the effectiveness of iodine. Phemerol, when used in 1:500 concentration, as a tincture, gave good results, the mortality being only 12%.

[7] That alcohol is able to kill only the least resistant bacteria is well known (McCulloch, E. C., *Disinfection and Sterilization* (Philadelphia, Lea and Febiger: 1945); Gershenfeld, L., *Bacteriology and Allied Subjects* (Easton, Pa., Mack Publ. Co.: 1945). The fact is not willingly accepted, however. Since the Council on Pharmacy and Chemistry of the American Medical Association has taken favorable note of Nungester and Kempf's results (10), it was surprising to find recently in the Journal a statement that "ethyl alcohol is an efficient disinfectant in certain concentrations . . . the most efficient strength is 70 per cent by weight" (*J. A. Med. A.*, **128**, 773, 1945).

The results of our tests (Table VI) confirmed those of Nungester and Kempf with regard to the reliability of iodine and the ineffectiveness of merthiolate. Of 58 mice in which iodine was used to disinfect the tail, only 2 died due to pneumococcus infection (3.4%); of 34 in which 1:1000 merthiolate was used,

TABLE V

Evaluation of disinfectants by "Infection-Prevention" test

DISINFECTANT	TEST ORGANISM				AVERAGE SURVIVAL TIME IN DAYS OF MICE WHICH DIED
	Pneumococcus		Streptococcus		
	Number of mice tested	Mortality, %	Number of mice tested	Mortality, %	
Aqueous phemerol, 1:1,000	53	92	30	96	3.1
Tincture phemerol, 1:1,000	94	72	31	90	3.8
Tincture phemerol, 1:500	68	12	38	64	4.1
Aqueous merthiolate, 1:1,000	56	100			
Aqueous merthiolate, 1:500			31	100	1.3
Tincture merthiolate, 1:1,000	68	62	27	96	2.8
Tincture mercresin, 1:1,000	57	26	31	94	3.4
Tincture mercresin, 1:500			27	89	3.5
Alcohol, 50%, acetone, 10%	51	69	18	100	2.7
Alcohol, 70%	30	63			
Aqueous iodine solution, 2%	56	5	29	48	3.2
Alcoholic iodine solution, 2%	27	0	28	36	5.1
Physiological salt solution	14	100			

TABLE VI

Infection-Prevention tests. Test organism is pneumococcus type I

DISINFECTANT IN AQUEOUS SOLUTION	NUMBER OF MICE USED	NUMBER OF MICE WHICH DIED (PNEUMOCOCCUS RECOVERED)	MORTALITY, PER CENT
DC-12 1:1,000	120	50	41.7
Metaphen 1:1,000	54	46	85.2
Merthiolate 1:1,000	34	33	97.0
Iodine in KI 2%	58	2	3.4
Physiological salt solution	143*	140	97.8

* The large number of controls for the entire series is due to the fact that the tests had to be done on small groups of animals consecutively during a considerable period of time, and it was necessary to test the virulence of the culture each time. We used 3 saline controls for each group of experiments.

33 died (97%). This percentage is the same as for the saline controls (140 of 143, or 97.8%). In 54 mice, 1:1000 metaphen[8] was used to disinfect the tail; of these 46 died (85.2%). In the case of DC-12 our first tests were made with a 1:1000 solution, for comparison with merthiolate and metaphen; of 120 mice, 50 died, or 41.7%. Our experiments with more concentrated solutions of DC-12 will be

[8] See footnote 6.

reported later, but it may be stated that by increasing the concentration tenfold (to 1%), we secured results comparable with those recorded here for 2% iodine.

TOXICITY

Although DC-12 is intended only for use as a disinfectant *in vitro* (it hemolyzes red blood cells, and therefore may not be used parenterally), a few preliminary tests for toxicity were made in rats, since such tests are prescribed in the circular published by the U. S. Navy for the examination of disinfectants (11).

Fifteen rats from 150 to 200 grams in weight were injected intraperitoneally with 0.2% DC-12, the amount injected being 1 ml. (containing 2 mg.) for each 200 grams of body weight. Except for 1 rat which died after 11 days with no gross changes at autopsy, all the rats remained well for a period of observation of 6 weeks. The rat tolerated the intraperitoneal injection of at least 2.8 mg. of DC-12.

In addition 12 rats were given DC-12 in their drinking water for a period of 2 months. Since they would not take a 1:1,000 solution at first, they were started on a 1:5,000 solution (16 days), then given 1:4,000 for 6 days, 1:3,000 for 14 days, and 1:2,000 for 10 days, before being given the 1:1,000 solution. They were kept on the 1:1,000 solution for 2 weeks but during the third week were found not to be drinking it. One animal died after 3 weeks and another was moribund, obviously as a result of dehydration, since autopsy yielded normal findings. The experiment was abandoned at this point and water substituted for DC-12. The remaining animals appeared normal within 48–72 hours. When they were autopsied a month later, together with 12 controls from the same litters, all appeared entirely normal.

It appears, therefore, that DC-12 can safely be used for disinfection of dental instruments.

SUMMARY

A quaternary ammonium salt, dimethyl benzyl lauryl ammonium chloride (DC-12), has been tested for its germicidal activity by the standard procedure for determination of phenol coefficients and by 2 of the newer procedures of testing disinfectants.

By the standard procedure, DC-12 was germicidal in a dilution of 1:30,000 with no organic matter present and in a dilution of 1:5,000 in the presence of 10% serum.

By the modified procedure *in vitro*, in which tests were made with several pyogenic cocci in the presence of both agar and serum, DC-12 was germicidal in a dilution of 1:1000.

Parallel experiments were made *in vivo* (mice) by the infection-prevention test of Nungester and Kempf, with the pneumococcus as test organism, of DC-12 in 1:1000 dilution as compared with 1:1000 concentrations of merthiolate and metaphen. The control solutions were 2% aqueous iodine and physiological saline. DC-12 in this concentration was more active than merthiolate and metaphen but less so than 2% iodine, which was nearly 100 per cent effective.

DC-12, even in 5% concentration, showed no fungicidal activity when tested against *Trichophyton mentagrophytes*, but a concentration of 0.1% killed spores of *B. cereus* and *B. subtilis* in 10 minutes.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH SCIENTIFIC PROCEEDINGS OF THE NEW YORK SECTION[1]

JUNE 7, 1945[2]

COMPILED BY FRANCES KRASNOW, PH.D., *New York Section*
Guggenheim Dental Clinic, New York, N. Y.

1. STATUS OF FLUORIDE THERAPY IN DENTISTRY. *Frances Krasnow, The Murry and Leonie Guggenheim Dental Clinic, New York, N. Y.* This resumé presents an inventory of the results thus far observed. Primarily, the discussion centers upon (1) effects of fluoride on the teeth of individuals living in areas with water supply containing it in varying concentration; (2) influence of topically applied fluoride solutions on dental caries incidence; (3) possibility that fluoride intake may affect the enveloping tooth structures; (4) introduction of fluoride in water supply as a public health measure for dental caries alleviation; (5) use of bone meal as fluoride source. Fundamentally the fluoride ion is extremely active. There are possibilities of danger both in administration as well as in accumulative effects on body tissues, notwithstanding recent evaluation to the contrary on the basis of height, weight, fracture studies or urine examinations. Also, fluoride administration cannot be considered a truly preventive measure. Greater ultimate benefit may be reaped from the energy and funds appropriated if cognizance were taken of the physiological needs that are stressed for early childhood and then forgotten. Data show that this deviation is rapid and may be the cause of the rapid rise in malfunction, both dental and general, as we proceed from childhood through adolescence to adulthood.

2. A STUDY OF THE TOPICAL APPLICATION OF POTASSIUM FLUORIDE IN CARIES PREVENTION. *Bion R. East, Daniel E. Ziskin, Lewis R. Stowe, Maxwell Karshan, Ellen P. Richardson,[3] School of Dental and Oral Surgery, Columbia University, New York, N. Y.* A preliminary report on the caries incidence in the newly erupted teeth of children subjected to repeated topical applications of potassium fluoride. Homologous teeth in the same individual served as controls, thereby nullifying such factors as diet, race, sex, etc. The investigating group consisted of an examiner, D. E. Ziskin, a custodian of records, Ellen P. Richardson, a treater, L. R. Stowe, a chemist, Maxwell Karshan, and a statistician, Bion R. East, who also planned the study. The examiner selected children with a pair of caries free teeth. Each child was referred with his examination record to the custodian of records who selected the tooth to be treated, first by lot and thereafter by alternation. Children were then sent with their treatment record in a sealed envelope to the treater who after applying the fluorine to the designated tooth returned the record in a sealed envelope to the custodian. Cases were

[1] The cost of publication of these proceedings is paid by the New York Section. Received for publication, June 25, 1945.

[2] Presiding—Daniel E. Ziskin.

[3] Guest.

recalled periodically, as near to 4 month intervals as possible for re-examination and treatment. Such examinations were made without benefit of any previous record, and recorded on a new form. Teeth were designated as x and y, the key being known only to the custodian. For the purpose of this report this key has not been released. At each visit teeth were treated with potassium fluoride 500 p.p.m. for two 3 minute intervals. The rubber dam was used wherever possible which was in the large majority of cases. Control teeth were handled in similar manner, distilled water being substituted for the fluorine solution. Of 48 children included in the original group 5 failed to report for the second examination and were dropped, 10 had 2 treatments and examinations, 11, three treatments and examinations and 22, four treatments and examinations. Included in this last group were 2 children who had 2 teeth each (treated and control). Of the 45 teeth in each group, as yet undifferentiated, the x group developed 13 cavities and the y group 11 cavities. Regardless of which group was treated the topical application of fluorine offered no protection from dental caries.

3. SYSTEMIC INTRODUCTION OF SODIUM IODIDE INTO CALCIFIED DENTAL TISSUES. *Irwin D. Mandel*[2] *and Lester S. Sarkady*,[3] *School of Dental and Oral Surgery, Columbia University, New York, N. Y.* Sodium iodide possesses ideal properties for this type of investigation. It is not a normal constituent of teeth; hence its detection after introduction into the experimental animal can be qualitative. Chemically it can be detected in quantity as low as 0.1 gamma. In high concentration it may be demonstrated histologically in ground section by precipitation with lead nitrate. Sodium iodide is excreted by the saliva so that it is possible to maintain a constant solution bathing the teeth as well as introducing it systemically. The substance was fed to 2 dogs in 1 gram capsules once a day for 30 days. At the end of this period the dogs were sacrificed and the teeth removed for histological and chemical examination. It was found that the feeding of sodium iodide in large quantities results in its deposition in the teeth, to a greater extent in the dentin than in the enamel and apparently concentrated in the innermost layer of the dentin. Grinding the enamel surface prior to feeding of the sodium iodide renders the enamel permeable to the saliva. An attempt was made to differentiate between salivary and systemic arrival of the iodide in the enamel by crowning several of the teeth before the feeding was made.

4. VITAL STAINING OF THE PULPS OF THE TEETH OF THE ALBINO RAT. *George Stein, School of Dental and Oral Surgery, Columbia University, New York, N. Y.* If certain electro-negative colloids like the dyes carmin, pyrrhol or trypan are injected into living animals, a selected group of cells accumulate these substances and are stained. Most of the cells which have the ability of vital straining belong to the reticulo-endothelial system or its derivatives. These cells play an important role in the defense organization of the body, and participate in all metabolic processes. If a 1% aqueous solution of trypan blue is injected subcutaneously into the albino rat in daily intervals, the animals acquire a blue color within a week. The vital stained cells are found in bone marrow, the spleen, the lymph nodes, the capillaries of the liver lobules, the cortex of the adrenals, the hypo-

physis, etc. Vital stained cells are seen in characteristic arrangement in the pulps of the continuously growing incisors but not in the pulps of the molars with completed growth. They are absent at the forming end of the incisors and increase gradually in numbers toward the incisal end where they become included in the central part of the tooth formed by calcified connective tissue. The experiment demonstrates 2 possible theories both emphasizing the ability of the dental pulp in producing defense or repair cells: (1) The cells collect chiefly at that part of the tooth where no dentin is formed but only calcified connective tissue separates the pulp from the tooth surface. An additional mechanism for repair and defense seems necessary. (2) Because no vital stained cells are found at the base of the tooth but in increasing number toward the narrowed end of the pulp, a second hypothesis may be suggested whereby the reticulo-endothelial system is functionally related to the process of reduction in cells and stroma which goes on at a rapid rate as the tooth erupts with a constant force exerted by the growing end.

5. THE HISTOLOGICAL DEMONSTRATION OF PHOSPHATASE IN THE DEVELOPMENT OF THE ENAMEL ORGAN. *Gerrit Bevelander and P. L. Johnson,*[1] *Department of Anatomy, New York University College of Dentistry, New York, N. Y.* In connection with the development of such complex structure as tooth there are still a number of unknown factors which have not been ascertained. This is particularly true in regard to the rôle of certain enzymes which are becoming increasingly important in developmental studies. For this and other reasons we have undertaken a study of the histochemical distribution of alkaline phosphatase in representative stages of the developing tooth. Considering first the dental lamina, it was observed that in the earliest state of development considerable amounts of phosphatase are present in the surface layers of the oral epithelium. In a somewhat more advanced stage there is a wider distribution in the oral epithelium and considerable amounts occur in the lamina itself. Moreover, the phosphatase appears to migrate from a lateral to a medial direction. As development progressed we ascertained that in the early bud stage the lateral two-thirds of the entire structure contains heavy concentrations of phosphatase. In the fully developed enamel organ we noted the characteristic appearance of phosphatase in most concentrated amounts within the stratum intermedium, in the stellate reticulum, and to a somewhat lesser degree in the inner and outer enamel epithelium. Appreciable amounts of this enzyme are present in the dental papilla and in the embryonic connective tissue capsule surrounding the enamel organ. In so far as our studies have gone, the rôle of alkaline phosphatase appears to be related to the histodifferentiation of the various tissues in the development of the enamel organ.

6. POSTERIOR GROWTH OF THE HUMAN MAXILLAE. *Moses Diamond, School of Dental and Oral Surgery, Columbia University, New York, N. Y.* A spreading phenomenon has been generally identified as part of the mechanism of sutural growth. In a previous study exception was taken to this view, and the "spreading phenomenon" was denied. Instead the suture keeps shifting its position in space in conformity with the direction of the remodeling of the bones adjacent to it.

[1] Guest.

At no time during the growth process do the peripheral surfaces of the bones adjacent to a suture make contact until the suture is ready to close. This is additionally understandable in terms of the three dimensional factor of growth. In a similar way the view is generally held that "spreading" during growth also occurs between bones not associated with sutures. This view is applied to the posterior growth of the maxillae. The explanation given is that the tuberosities of the maxillae are buttressed against the pterygoid processes of the sphenoid and as posterior growth of the maxillae occurs, the entire maxillae are pushed forward. This anatomical relation of the maxillary tuberosities lying adjacent and anterior to the pterygoid plates of the sphenoid does in fact exist in the adult stage of development. The conclusion is drawn on the false assumption that this relationship also exists during the growing stages and that the pterygoid processes remain as a landmark of fixed relationship to the maxillary tuberosities. The evidence shows that neither of these is true. Instead the maxillae grow posteriorly into the sphenomaxillary space, laterally and posteriorly to the pterygoid plates. The pterygoid plates are steadily remodelled posteriorly and laterally in synchronous relation to the posterior growth of the maxillae, lagging a step behind until the adult stage is reached. When the posterior growth of the maxillae is completed, the pterygoid processes then complete their remodelling and assume a position directly posterior to the maxillary tuberosities. There is therefore, no evidence of forward or anterior spreading of the maxillae during their posterior growth.

7. EVOLUTION OF ORTHODONTIC THEORY AND PRACTICE. *Samuel Hemley, New York University College of Dentistry, New York, N. Y.* The evolution of orthodontic appliances is correlated with the associated theories regarding their use. In this process it is shown that the application of excessive force in the earlier years of orthodontic practice led to the introduction of other appliances to compensate for the ill effects of this excessive force. Each new appliance in turn presented additional problems and the attempts to compensate for these new problems led the profession further from the solution of the original problem which arose from the application of excessive force. The conclusion arrived at is that the application of proper forces, used in accordance with the known reactions of tissues of these forces, eliminated the need for the more complicated applicances.

8. TOTAL ERYTHROCYTE, LEUCOCYTE AND DIFFERENTIAL WHITE CELL COUNTS OF BLOOD IN CHRONIC PERIODONTAL DISEASE. *Eli H. Siegel,*[4] *U. S. Navy Dental Clinic, Navy Yard, New York, N. Y.*[5] One hundred young adult males with either chronic marginal gingivitis, chronic Vincent's gingivitis, or extensive alveoloclasia were the subjects of this study. A group of 75 individuals without periodontal disease was used as a control. Those with periodontal disease had an average lower total erythrocyte count (4.5 $\pm$ 0.44 million/c.mm.) than the control group (5.0 $\pm$ 0.33 million/c.mm.). Statistical evaluation of this differ-

[4] Samuel A. Rosenbaum, U. S. N. R., provided technical assistance in this study.

[5] The material in this abstract should be construed only as the personal opinion of the writer and not as representing the opinion of the Navy Department, officially.

ence indicates significance. The total average leucocyte counts for both groups fell within the accepted normal range and showed no significant group difference. Neither group showed a characteristic distribution in the differential leucocyte counts. The depression in the number of erythrocytes is apparently secondary to the presence of periodontal disease, since arrest or cure of these pathological processes in individual cases results in the elevation of the erythrocyte count to normal or high normal levels. In several instances, counts were made during an initial acute attack of Vincent's gingivitis and the patient neglected to appear for follow-up treatment. The acute process then subsided into a chronic state and the patient upon reappearance for treatment several weeks or months later, showed lowered erythrocyte counts which reverted to normal levels upon cure of the chronic periodontal disease. This finding indicates that chronic periodontal disease can be a cause of physical debility and reduced efficiency. It also suggests that study of changes in the erythrocyte count during treatment of periodontal disease may be used as an objective laboratory guide in judging the progress or success of treatment. The fact that chronic periodontal disease is to be considered a possible contributing factor or cause of mild anemia is also indicated.